HUMAN AND ECOLOGICAL RISK ASSESSMENT OF NONYLPHENOL POLYETHOXYLATE-BASED (NPE) SURFACTANTS IN FOREST SERVICE HERBICIDE APPLICANTS

Human and Ecological Risk Assessment of Nonylphenol Polyethoxylate-based (NPE) Surfactants in Forest Service Herbicide Applications

David Bakke, USDA Forest Service
Pacific Southwest Region (Region 5)
Pesticide Use Specialist
May 2003

TABLE OF CONTENTS

The following individuals provided peer reviews of this report:

Dr. Patrick Durkin reviewed this document, under contract to the USDA Forest Service. Dr. Durkin also authored Appendix 2 - Risks of Ethylene Oxide in NPE Surfactants. Dr. Durkin is co-founder and principal scientist of Syracuse Environmental Research Associates (SERA), Inc. Prior to founding SERA in 1991, Dr. Durkin was employed at Syracuse Research Corporation, serving as director of the Life and Environmental Sciences Division and later as director of the Chemical Hazard Assessment Division (1972 to 1991). Dr. Durkin is a charter member of the American College of Toxicology, a charter member of the Society of Risk Analysis, a member of the American Association for Advancement of Science and the New York Academy of Science and is an Adjunct Professor at the SUNY College of Environmental Science and Forestry (1985-date). Dr. Durkin has conducted numerous risk assessments and risk assessment method development tasks for the USDA, U.S. EPA, and CDC/ATSDR. In recognition of his contributions to the development of the U.S. EPA's Ambient Water Quality Criteria, Dr. Durkin was given the U.S. Environmental Protection Agency Award for Special Achievement. Dr. Durkin has been appointed a consultant to the U.S. EPA Science Advisory Board (SAB) and is a member of the FQPA Science Advisory Panel (SAP) on cumulative risk. Dr. Durkin has also served as a reviewer of the risk assessment methods used by the Agency for Toxic Substances and Disease Registries (ATSDR), the Dutch Health Council, and for the journals Toxicology and Industrial Health as well as Risk Analysis.

Dr. Gary L. Diamond reviewed this document. Dr. Diamond is a Senior Scientist at the Environmental Science Center of Syracuse Research Corporation. He is a member of the American Association for Advancement of Science, New York Academy of Science, and Society of Toxicology. Dr. Diamond is an adjunct profession at SUNY College of Environmental Science and Forestry (1997-date) and an adjunct associate Professor in toxicology at the Department of Environmental Medicine, University of Rochester (1992-date). Dr. Diamond has served on advisory committees for the National Research Council, the U.S. EPA Science Advisory Board, and the U.S. Nuclear Regulatory Commission, as well as a peer-reviewer for the National Institute of Occupational Safety and Health. Dr. Diamond currently heads several research projects with EPA to develop exposure-biokinetic models for use in risk assessment and to develop methods for the consistent integration of bioavailability information into quantitative risk assessment. Dr. Diamond currently serves as SRC Program Manager of a contract that supports the EPA OERR lead risk assessment methodology and model development programs, and he is deputy Program Manager of a contract that supports the ecological and human health risk assessments programs of EPA Region 8.

Executive Summary

The primary active ingredient in many of the non-ionic surfactants used by the Forest Service when applying herbicides is a component known as nonylphenol polyethoxylate (NPE). NPE is found in these commercial surfactants at rates varying from 20 to 80%. NPE is formed through the combination of ethylene oxide with nonylphenol. Nonylphenol (NP) is a material recognized as hazardous by the United States Environmental Protection Agency (U.S. EPA) (currently on U.S. EPA's inerts list 1). Both NP and NPE exhibit estrogen-like properties, although they are much weaker than the natural estrogen estradiol.

Human Health Risk Assessment

Based on the estimated levels of exposure and the criteria for acute and chronic exposures, there is no evidence that typical exposures to NP9E-based surfactants will lead to dose levels that exceed the level of concern. For workers, only the upper levels of operational exposure result in estimates of absorbed doses that exceed the derived RfD by a modest amount. It is unlikely that any worker would be utilizing such high levels of NP9E-based surfactants on a chronic basis; the high rate in this scenario is uncommonly used in the Forest Service. However, this does point out the need for good industrial hygiene practices when utilizing high levels of NP9E-based surfactant.

Given the low hazard quotients for accidental exposure, the risk characterization is reasonably unambiguous. None of the accidental exposure scenarios exceed a level of concern. While the accidental exposure scenarios are not the most severe one might imagine they are representative of reasonable accidental exposures.

For members of the general public, the upper limits for hazard quotients for chronic exposures are below a level of concern and the risk characterization is relatively unambiguous. Based on the available information and under the foreseeable conditions of application, there is no route of exposure or scenario suggesting that the general public will be at any substantial risk from longer-term exposure to NP9E-based surfactants.

For the public, acute or accidental exposure scenarios involving consumption of contaminated water, consumption of contaminated vegetation, or subsistence consumption of fish represent some risk of effects. None of the other acute exposure scenarios represent a risk of effects to the public from NP9E exposure. At typical rates of application, the drinking of contaminated water after a spill could present a risk of subclinical effects to the liver and kidney. The exposure scenario for the consumption of contaminated water is an arbitrary scenario: scenarios that are more or less severe, all of which may be equally probable or improbable, easily could be constructed. The consumption of contaminated vegetation also represents a risk of clinical effects at the high application rates only. At the typical rate of application, the risk is considered acceptable. Nonetheless, this and other acute scenarios help to identify the types of scenarios that are of greatest concern and may warrant the greatest steps to mitigate. For NP9E, such scenarios involve oral rather than dermal exposure.

Irritation and damage to the eyes can result from exposure to relatively high levels of NP9E -i.e., placement of NP9E directly onto the eye - and repeated exposures to undiluted NP9E-based surfactants can lead to skin sensitization. From a practical perspective, eye irritation and skin sensitization are likely to be the only overt effects as a consequence of mishandling NP9E. These effects can be minimized or avoided by prudent industrial hygiene practices during the handling and application of NP9E-based

surfactants.

There is the potential for exposures to other man-made and natural estrogen-like compounds. A consideration of potential cumulative effects is limited because of a lack of comprehensive information on the skin absorption kinetics of NPE in mammalian systems. Contributions to background exposures to other xenoestrogens from exposure to NPE may be negligible depending upon the background exposures, lifestyles, absorption rates, and other potential natural or man-made chemical exposures that are used to determine overall risk to environmental xenoestrogens.

U.S. EPA is completing testing protocols and setting priorities for testing for endocrine effects, as a result of the passage of the Food Quality Protection Act of 1996. As the body of research on endocrine effects continues to grow through this effort and others, the Forest Service will review new studies to determine whether any of the conclusions in this paper should change.

Ecological Risk Assessment

Based on the expected chronic exposure levels, there is little risk to terrestrial wildlife at any application rate considered in this risk assessment. With the typical application rates, two scenarios represent a slight risk of effects to mammals: direct spray to a small mammal (assuming the skin affords no protection) and consumption of contaminated vegetation by a large grazing mammal, such as a deer. None of the other acute exposures at the typical rates of application represent a risk of effects to terrestrial wildlife. At the highest application rates, acute exposures from the consumption of contaminated vegetation present a risk of effects, assuming 100% of consumed vegetation is contaminated. If we assume the skin is not a barrier at all (100% absorption), then the direct spray also provides a risk of effects at the highest application rates.

For aquatic species, the duration of any exposure would be short; the compounds of concern are broken down and their concentration reduced through dilution, as well as binding of the compounds to stream sediments.

The ambient levels of NP9E assumed to be present from normal operations would be protective of all aquatic organisms. These assumed levels are at least 30 times lower than the 1,000 ppb protective level for NP9E, and at least 15 times lower than the assumed acute protective level for NP. Given the chronic exposure to NP1-2EC of 7 ppb (0 to 14 ppb range), there should be no chronic risk to aquatic species.

Both the overspray and the spill scenarios involve levels of NP9E that could represent a risk of toxic effects. The overspray scenario exceeds the acute NP9E threshold by a factor of 1.5 (central estimate), up to a factor of 4.9 (highest rate). With a spill, the NP9E threshold is exceeded by a factor of 6.1 (central estimate), up to a factor of 15.1 (highest rate). In a stagnant small pond or stream reach, there could be effects seen to aquatic organisms. However in a live stream, the more realistic scenario would be a short-term pulse of concentrated NP9E moving downstream, mixing with water and being broken down into NP1-2EC and/or partitioning into sediments. The effects of a short pulse should be minor on aquatic organisms as the short exposure time would result in lower doses than are discussed here.

For aquatic plants, similar conclusions are reached; the normal applications should not represent a risk of effects, either through acute or chronic exposures, while the spill or overspray scenarios do represent a risk of effects.

1. Introduction

There has been concern expressed about the potential for surfactants containing nonylphenol polyethoxylate (NPE) to cause endocrine disruption as a result of their use on herbicide projects in Region 5 of the Forest Service. There have also been concerns expressed about the acute toxicity to aquatic organisms of the chemical components and breakdown products of NPE-based surfactants. This paper is meant to develop an understanding of the issues and to assist biologists and others in analyzing the risk of potential health and environmental impacts resulting from the use of surfactants containing NPE.

That these surfactants contain NPE is a cause for concern, since the raw material used to make NPE, nonylphenol (NP), as well as NPE itself, has been shown to exhibit estrogenic properties in lab tests. NP is also highly toxic to many aquatic organisms at low concentrations (and is on the U.S. EPA's Inert List 1)[1].

2. Program Description

2.1 Overview

NPE-based surfactants are made up primarily of a nonylphenol polyethoxylate (NPE) ingredient, which puts it in a broad class of chemicals known as alkylphenol ethoxylates (APE's). Given some conservative assumptions about use of NPE surfactants, the total use in the Forest Service would be less than 0.2% of the production of NPE surfactants in the United States. This would indicate that nationally, the Forest Service contributes little to the potential load of NPE and its metabolites in the United States. However, the use of NPE based surfactants could result in local increases in NPE in the environment.

Commercial NPE-based surfactants are generally mixed with herbicides and water carriers at dilution rates of 0.25% to 2.5%. For the purposes of this analysis, a typical dilution will be 1%, with a range of 0.25% to 2.5%.

2.2 Chemical Description and Commercial Formulations[2]

NPE-based surfactants are made up primarily of a nonylphenol polyethoxylate (NPE) ingredient, which puts it in a broad class of chemicals known as alkylphenol ethoxylates (APE's). This class includes octylphenol polyethoxylate (OPE). NPE and OPE are widely used materials in the U.S. (industrial uses,

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[1] U.S. EPA has divided pesticide inert ingredients into 4 basic lists. List 1 is made up of compounds that U.S. EPA has identified as being of significant toxicological concern. List 2 contains compounds of potentially significant toxicity that are a high priority for testing. List 3 contains those compounds, which U.S. EPA has no basis for placing on the other lists. List 4 contains inerts of minimal concern. In Region 5, we have endeavored to avoid the use of products with ingredients on Lists 1 or 2 unless a risk assessment has been completed and the risk has been assessed as acceptably low. The complete listing is available on-line at http://www.epa.gov/opprd001/inerts/lists.html.

[2] Manufacturers are not required to disclose ingredients in surfactants specifically, as they are protected as Confidential Business Information (CBI). Manufacturers can describe the active ingredients in generic chemical terms; the same generic term could represent different chemistries. For the purpose of this analysis, several surfactants are assumed to contain NPE based on these generic terms.

detergents, cosmetics, shampoos, surfactants, spermicides), with NPE making up the majority of uses in commerce (about 80% of total APE's) (APERC 1999a). When used in products designed for human exposure (cosmetics, spermicides, etc), NPE is often referred to as nonoxynol. Structurally, nonoxynols and NPEs are the same, as long as the number of ethoxylate groups is identical. Much of the human and mammalian health data in this report comes from studies involving nonoxynols with 1-30 ethoxylate groups. Octoxynols are the same as OPEs.

Based on a recent market analysis report on nonylphenol, annual demand in the United States for nonylphenol in 2000 was in the range of 240 million pounds (Chemical Market Reporter 2001). About 80% of this NP was used to produce industrial and institutional surfactants and liquid detergents (NPE) with the remainder being used primarily for the production of rubber, plastics and lubricating oils. Based on the latest pesticide-use report of the Forest Service for 2001, 186,000 acres were treated with herbicides, algicides, or plant growth regulators. If each of these acres treated also was treated with an NPE surfactant at the typical application rate of 1.67 lbs/acre, the total use in the Forest Service would be less than 0.2% of the production of NPE surfactants in the United States. This would indicate that nationally, the Forest Service contributes little to the potential load of NPE and its metabolites in the United States. However, the use of NPE based surfactants could result in local increases in NPE in the environment.

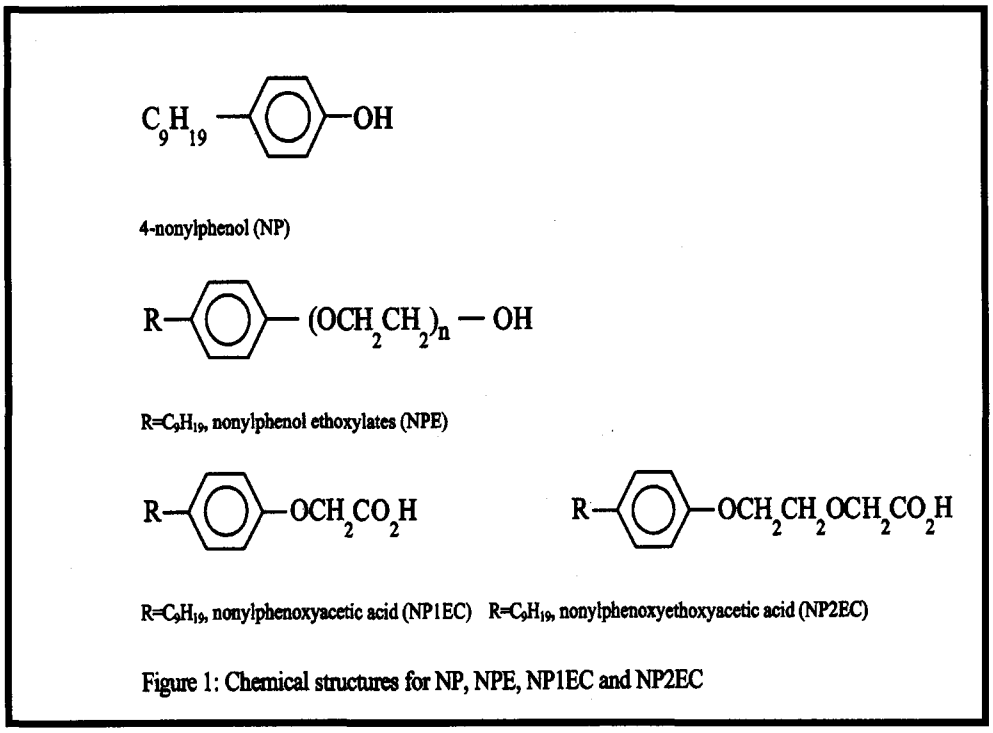

Figure 1: Chemical structures for NP, NPE, NP1EC and NP2EC

One important concept to understand with NPE and OPE is that these terms describe a large number of compounds. In the production of NPE, various numbers of ethoxylate groups are attached to a nonylphenol (NP) molecule, through a reaction of NP with ethylene oxide. NP is a molecule made up of a nine-carbon alkyl group (nonyl) joined to a phenol. The properties of the particular NPE depend upon the number of ethoxylate groups that are attached, and this number can vary from just a few, up to about a hundred. The most common NPE used in surfactants with pesticides is a mixture that has, as a

majority, 8-10 ethoxylate groups attached.[3] But it is important to understand that there is a bell-shaped distribution curve around 9 ethoxylate groups in such a mixture, and that other longer and shorter-chain NPEs also exist in the mixture. An average of 8-10 ethoxylate groups makes these surfactants highly water-soluble. With less than six or seven ethoxylate groups, the material is more water insoluble (APERC 1999a).

Examples of synonyms for nonylphenol polyethoxylate:

Nonoxynol
Polyoxyethylene nonyl phenyl ether
Polyethylene glycol nonyl phenyl ether
Nonylphenoxypolyethoxyethanol
Polyoxyethylene nonylphenol
Nonylphenyl polyethylene glycol
Nonylphenol polyoxyethylene ether

Analysis of NP9E surfactants has shown various distributions of the longer and shorter chain NPEs around the peak at NP9E. In most studies that analyze the components, the short-chain NPEs (NP1E, NP2E, NP3E) are lumped together in one group, since they exhibit similar properties. The short-chain NPEs make up anywhere from 0.58% to 5% of the total NP9E mixture (Ahel et al 1994a; An-Ex Analytical Services Ltd., 1995b (unpublished); John et al 2000; Mann, Bidwell 2001; Mann, Boddy 2000; Miles-Richardson et al 1999). In five of these studies, NP is not detectable. In one study, NP is indicated as being present although the method of analysis is not detailed in the study, and the NP is lumped with NP1E and NP2E with all three indicated as being 0.58% of the total (Miles-Richardson et al 1999). . In an unpublished lab report (An-Ex Analytical Services, Ltd. 1995d, unpublished) using a sensitive method of analysis, there was no NP or NP1-3E detected in nonoxynol-9. Based on a personal communication with Dr. Carter Naylor (Naylor 2001), NP9E should have less than 0.1% NP; numbers he has measured have been in the 50-500 ppm range [50 ppm = .005%, 500 ppm = .05%]. The short chain ethoxylate, NP1E is slightly more common, but still should be less than 1% of the total

There are several commercial surfactants available for use in Region 5 that are assumed based on NP9E. Several of the commercial brand names used in recent years are R-11® (Wilbur-Ellis Company), Activator 90® and X-77® (Loveland Industries) and Latron AG-98 (N) Intermediate® (Dow Agrosciences). These are all non-ionic surfactants, often added to foliar active herbicides, such as glyphosate, triclopyr, and clopyralid. There are some surfactants that contain OP9E, such as Latron AG-98®, but they are not as commonly used as NP9E-based surfactants.[4]

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[3] In this paper, the average number of ethoxylate groups and the APE will be combined into a standard shorthand. For example NP9E will represent a nonylphenol polyethoxylate with an average of 9 ethoxylate groups. Unless otherwise stated, NP9E will represent the average surfactant ingredient, even though these surfactants may contain an average of 8 to 10 ethoxylate groups.

[4] Mention of brand names and manufacturers is for illustrative purposes only and does not imply recommendations for use or endorsement of these products by the United States Department of Agriculture.

In addition to NP9E, these surfactants also commonly include an alcohol (such as butyl or isopropyl alcohol), making up about 10% of the mixture; a silicone defoamer (about 1% of the mixture); and water. The NP9E makes up the majority of the formulation, often around 80% of the formulation.

2.3. Application Methods

The most common method of ground application for NPE-based surfactants involves backpack and boom spray applications of herbicide tank-mixes. Application crews may treat up to shoulder high brush, which means that chemical contact with the arms, hands, or face is plausible. Boom sprays are more typically used in rights-of-way management. NPE-based surfactants can be part of herbicide mixtures applied via aerial applications, either with fixed –wing or helicopter.

There are specific brands of NPE-based surfactants that are registered for aquatic and/or near-riparian uses.

2.4. Mixing and Application Rates

Commercial NPE-based surfactants are generally mixed with water-soluble herbicides and water carriers at dilution rates of 0.25% to 2.5%, rather than in terms of pounds per acre. For the purposes of this analysis, a typical dilution will be 1%, with a range of 0.25% to 2.5%. This equates to an application rate of 0.8% NP9E active ingredient (ai), with a range of 0.2% to 2% ai. For ground-based herbicide applications, application rates of the herbicide mixture range from 10 to 40 gallons per acre (gpa), with 25 gpa representing the typical application in the Forest Service. Therefore, the surfactant active ingredient is applied at a rate of 0.20 gpa (25 gpa x 0.008) with a range of 0.020 to 0.8 gpa ai. This is equivalent to a pound per acre rate of 1.67 lbs/acre (range of 0.167 to 6.68 lbs/acre).

3. Human Health Risk Assessment

3.1. Hazard Identification

3.1.1. Overview

NP4, 5,6,9E are classified as slightly toxic to practically non-toxic to mammals and are placed in EPA toxicity category III or IV[5]. In comparison with NP4, 5, 6, and 9E, the acute toxicity of NP is somewhat higher.

Based on subchronic and chronic testing, it appears that the liver and kidney are the organs most likely to be affected by exposures to NPE and NP. NP and NPE have been determined to be weakly estrogenic in both *in vitro* and *in vivo* tests involving aquatic and terrestrial organisms. Non-reproductive effects appear to be the more sensitive endpoint. The NOAEL for chronic effects is assumed to be 10 mg/kg/day based on liver and kidney effects in rats. This NOAEL would be protective of teratogenic and reproductive effects.

No evidence of carcinogenicity was reported in 2-year chronic oral toxicity studies of NP9E with rats and dogs. NP9E does not appear to be immunotoxic or neurotoxic at doses considered protective of kidney or liver effects.

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[5] US EPA toxicity categories are defined in Federal regulations (40 CFR 156.10).

NP9E is considered minimally to severely irritating to rabbit skin and moderately to severely irritating to rabbit eyes. Contact dermatitis and contact photosensitivity has been reported in humans following exposure to NP6E, NP10E, and NP12E in consumer products.

Based on one study of NP9E, it appears to be rapidly metabolized and excreted. After injection of NP9E into rats, NP9E was completely metabolized and these metabolites were primarily excreted in feces and secondarily in urine. Analysis of urinary metabolites indicated the presence of highly polar neutral and acidic species.

Ethylene oxide has been found in NP9E at low levels, up to 12.2 mg/L (ppm), in the unreacted form as a residual from the manufacturing process. Ethylene oxide has been described as a probable human carcinogen with sufficient evidence in experimental animals to support a finding as a carcinogen; it is also a mutagen. 1,4-dioxane has also been found as an impurity in NP9E at low levels (<4.5 to 5.9 ppm) and has also been classified as a carcinogen.

U.S. EPA is completing testing protocols and setting priorities for testing for endocrine effects, as a result of the passage of the Food Quality Protection Act of 1996. As the body of research on endocrine effects continues to grow through this effort and others, the Forest Service will review new studies to determine whether any of the conclusions in this paper should change.

3.1.2. Acute Toxicity

For NP9E, oral acute toxicities (LD_{50})[6] in rats range between 1410 and 5600 mg/kg; in rabbits, mice, and guinea pigs the LD_{50} ranges between 620 and 4400 mg/kg (Environment Canada 2001a). For NP5E the acute oral toxicity in rats (oral LD_{50}) ranged from 3500-4500 mg/kg; for NP6E in rats, the acute oral toxicity equals 1980 mg/kg (CTFA 1979a, 1979b, unpublished). For NP4E, the LD_{50} values in the rat vary between 4290 and 7400 mg/kg (Environment Canada 2001a). These values would classify NP4, 5,6,9E as slightly toxic to practically non-toxic to mammals and place them in EPA toxicity category III or IV.

In comparison with NP4, 5, 6, and 9E, the acute toxicity of NP is somewhat higher, with LD_{50} values of 580 to 1,620 mg/kg (Environment Canada 2001a). These levels would classify NP as slightly toxic to practically non-toxic to mammals and place it in EPA toxicity category III or IV.

3.1.3. Subchronic or Chronic Systemic Toxic Effects

Based on subchronic and chronic testing in rats and beagles, it appears that the liver and kidney are the organs most likely to be affected by exposures to NPE and NP. At the cellular level, there is some indication that NP affects the mitochondria, although effects to mitochondria are not seen with NPEs (Bragadin, et al 1999). NOAELs for NP9E subchronic and chronic exposure in rats and beagles generally ranged from 10-40 mg/kg/day.

3.1.3.1 – NP9E

There are three unpublished 90-day subchronic feeding studies in rats involving NP9E discussed in Smyth and Calandra 1969. In a 90-day subchronic feeding study in Charles River rats, NP9E was fed in

[6] The LD50 is a standard measure of acute toxicity in toxicological testing. It is defined as the dose of a chemical calculated to cause death in 50% of a defined experimental animal population over a specified observation period.

the diet at doses of 1,250, 250, 50, and 10 mg/kg/day (Mellon Institute, 1959-1965, unpublished). At the 50 mg/kg/day dose level, there was a decrease in relative kidney weight, but this effect was not seen at higher or lower doses. There was also a slight decrease in liver polysaccharides at 50 mg/kg/day, which is considered the LOAEL for this study. More significant effects to the liver and kidneys were seen at the upper two dose levels. In another 90-day subchronic feeding study, Sherman Wistar rats received diets containing 0.01% to 5.0% NP9E (Shelanski, M.V., 1960, unpublished). Rats receiving diets of 2.5% or 5% NP9E (approximately >225 mg/kg/day) were emaciated, had scanty body fat and poor organ development, interpreted as being due to partial starvation. No histopathological changes indicating toxic effects were seen. Red and white blood cell counts, differential white cell counts, and hemoglobin remained normal. The only statistically significant effect was retardation of weight gain at 0.64% and higher NP9E in the diet (approx >65 mg/kg/day). Based on weight loss, the NOAEL for this study was at 0.16%, or approximately 17-20 mg/kg/day. In a third study in rats, NP9E was added to feed at levels of 0.1%, 0.3% and 1.0% (Dow, 1960, unpublished data). No effects were seen at 0.1% (20 mg/kg/day). At 0.3% (66 to 75 mg/kg./day) there was an increase in relative liver weight in both males and females. At the highest dose rate (1.0%, or 220 to 240 mg/kg/day), there was an increase in relative liver, kidney and spleen weights.

In 90-day subchronic studies in Sprague-Dawley rats, the oral toxicity of NP6E resulted in a NOEL of 40 mg/kg/day based on increased liver to body weight ratios seen at 200 mg/kg/day in both males and females and increased relative kidney weights in males at 200 mg/kg/day (Industrial Bio-Test Laboratories, 1963a, unpublished data). There were decreases in weight gain at the highest dose tested (1000 mg/kg/day), but not seen at 200 mg/kg/day (*ibid*). There were no effects noted to blood or urine chemistry (*ibid*). There were no gross pathologic changes or histopathological changes in any organ examined (*ibid*). In Smyth and Calandra, 1969, the authors state that the increased liver weights are attributed to an increase in liver tissue resulting from increased enzyme activity in metabolizing the NP6E, since no histopathology was found in liver sections.

In an unpublished study, beagles were exposed to NP9E in their diets at rates of 0.04%, 0.64% and 5.0% for 90 days (Shelanski, 1960. Unpublished). There were no histopathologic changes indicating toxic effects, but there were changes in the livers to all dogs, including controls, indicating a parasitic infestation. Red and white blood cell counts, differential white blood cell counts, and hemoglobin remained normal. Although the data show differences in relative organ weights, the only statistically significant effect was a decrease in weight gain seen at the two higher doses (approximately 170 and 1,200 mg/kg/day), related to a decrease in voluntary food intake. The NOAEL for this study was 0.04% NP9E in the food (approximately 11-14 mg/kg/day).

In an unpublished 90-day subchronic test, beagles were dosed with NP4E at rates of 40, 200, and 1,000 mg/kg/day via gelatin capsule (Industrial Bio-Test Laboratories. 1963c. Unpublished, cited in Smyth and Calandra 1969). Relative liver weight was increased at 200 mg/kg/day, with emesis evident during the first 3 weeks. No effects were seen at 40 mg/kg/day to any organ examined (including heart, liver, lungs, spleen, brain, and kidneys). In another study by the same laboratory (Industrial Bio-Test Laboratories, 1963b), beagles were exposed to NP6E with the same dosing regimen; occasional emesis was noted during the first 3 weeks at the 200 mg/kg/day dose level, but relative liver weight was not affected at this dose. There was a slight increase in relative liver weight in females at 1,000 mg/kg/day, but this effect was not considered statistically significant. Gross and histopathologic exams didn't indicate any changes and there were no abnormalities in hematological studies, blood chemistry, urinalysis, or liver function.

Intraperitoneal injection of NP9E at 50 mg/kg/day for 5 days in rats produced morphological and biochemical changes in the liver (Chvapil et al 1986). There was an increase in the content of collagen in the liver, as well as an increase in cellularity, and an increase in the amounts of rough endoplasmic reticulum. None of these changes were seen in the lungs. Vaginal instillation of NP9E at 50 mg/kg/day for 5, 10, 15, and 20 days into female rats produced morphologic and biochemical changes in the liver (an increase in collagen, lesions, as well as an increase in rough endoplasmic reticulum) and biochemical changes in the kidneys (an increase in DNA content and total hydroxyproline) (Chvapil et al, 1986).

Smyth and Calandra, 1969, briefly describe an unpublished study where NP9E was administered in the diet of Carworth-Elias rats over a 2-year period. The NP9E was added to their food at rates of 0.03%, 0.09%, and 0.27%. There is insufficient information presented to determine how these doses translate into mg/kg/day. There was no difference between control and treated rats in any observation made – food consumption, mortality, life span, extraneous infections, liver and kidney to body weight ratios, body weight gain, maximum weight attained, red blood cell count, incidence and location of neoplasms, histopathologic lesions.

Another unpublished study is described in Smyth and Calandra, 1969, in which Sprague-Dawley rats were exposed to NP4E in the diet for 2 years. Dose rates were at 40, 200, and 1,000 mg/kg/day. There was no mortality or behavioral differences associated with any dose. At the two highest doses, females had gained less weight at 12 months, but did not differ from controls at 24 months; males saw this same effect at the highest dose only. Differences in weight gain were attributable to poor palatability of the diet. The only other dose-related effect noted was a slight elevation in relative liver weights in both sexes at an intake of 1,000 mg/kg/day. Microscopically the livers were normal; hence the response was not considered adverse.

In an unpublished 2-year chronic exposure study in beagles described in Smyth and Calandra, 1969, the beagles were exposed to NP9E in the diet at rates of 0.03%, 0.09%, and 0.27%. The only effect attributable to the treatment was an increase in relative liver weight at the highest dose rate (equivalent to a dose of 88 mg/kg/day), with a corresponding NOAEL of 0.09% (approximately 28 mg/kg/day). .

In humans, exposure to NP9E has been studied primarily as it relates to its use as a spermicide (nonoxynol-9) and has involved vaginal applications. Although this method of exposure is not pertinent to this risk assessment, these are the few studies that have looked at NP9E exposure in humans. There are four studies described in Johnson, 1999 where women were exposed intravaginally to NP9E for 14 days at levels ranging from approximately 1.5 to 12 mg/kg/day (assuming an average body weight of 64 kg) (Chvapil et al. 1982; Malyk, 1981; Niruthisard et al. 1991; Roddy et al. 1993). No indication of absorbed dose rates is discussed. In Malyk 1981, 30 women were exposed to 125 mg/day of NP9E intravaginally for 14 days (approximately 2 mg/kg/day). Blood chemistries were taken prior to exposure and post-exposure. No treatment related differences were found. An evaluation of liver function, using serum enzymes as an indirect measure, showed no effects (*ibid*). In Niruthisard et al 1991, 14 women were exposed to 150 mg NP9E four times a day (once per hour over 4 hours) for 14 days (a daily dose of approximately 9 mg/kg/day). Common effects seen were vaginal irritation, disruption of the epithelium, redness of the epithelium, and bleeding. One subject experienced a severe edematous reaction (with bleeding) of the cervix (*ibid*). No effects were seen to hepatic functions or hematologic parameters, with the exception of the study by Chvapil et al (1982), which showed a drop in serum cholesterol. In a study using adult rats, a single intravaginal dose of NP9E (50 mg/kg) caused inflammation and necrosis of mucosal cells in the vagina (Tryphonas and Buttar 1982).

3.1.3.2. - NP

A 28-day subchronic study in rats exposed to NP, showed a male LOEL of 25 mg/kg/day based on increased relative liver weight, and a female NOEL of 400 mg/kg/day (highest dose tested) (Richards, 1989 unpublished, cited in Environment Canada 2001a).

In a 90-day subchronic study, Sprague-Dawley rats exposed to NP at doses of 0, 200, 650, and 2000 ppm in feed had a NOEL of 650 ppm (50 mg/kg/day) based on small decreases in body weight and food consumption, a small increase in relative kidney weights and a decrease in renal hyaline in males at highest dose (Cunny et al 1997). The authors considered the kidney weight changes to not be toxicologically significant as the changes were within historical lab control values. The changes to renal hyaline were not considered toxicologically relevant to humans, as renal tubular hyaline is associated with rat-specific protein (alpha-2u-globulin). There were no effects to reproductive organs, no changes in estrous cycles, or any changes to sperm at the highest dose (129 to150 mg/kg/day) (*ibid*). The decrease in renal hyaline globules seen at 2,000 ppm may be an indirect effect of the estrogenic properties of NP. The male rat develops these hyaline globules in response to the large amounts of alpha-2u-globulin (a protein synthesized in the liver and excreted with the urine) filtered by the male kidney; the droplets contain the alpha-2u-globulin. The female rat liver does not produce as much alpha-2u-globulin as the male, hence there are rarely hyaline globules found in the female rat kidney. Exposure to androgens, such as testosterone have been shown to result in an increase in the hepatic production of alpha-2u-globulin in male rats (Murty et al 1987), while the opposite effect can be achieved by exposure to estrogens, such as estradiol (Roy et al, 1977). Therefore a decrease in the incidence of hyaline globules in male rats seen in Cunny et al 1997 may reflect some type of hormonal influence caused by exposure to NP. However, even if the changes to renal hyaline seen in Cunny et al 1997 can be considered to be an indicator of estrogenic effects, this effect was only seen at the highest dose (129 mg/kg/day average dose for males at 2,000 ppm), indicating a NOAEL of 45 mk/kg/day (the average male dose at 650 ppm).

In a two-generation exposure study in Sprague-Dawley rats exposed to NP via oral gavage at doses of 2, 10, and 50 mg/kg/day, relative liver and kidney weights increased in males exposed to the highest dose but not in females (Nagao et al, 2001). In the F0 generation, in addition to weight increase in liver and kidney in males, there was also an increase in relative weight of the male brain, lungs, thyroid, and pituitary gland and a decrease in the relative weight of the thymus. In females there was a relative decrease in the weight of the ovaries in both the F0 and F1 generations at 50 mg/kg/day. Histopathologic changes included centrilobular hypertrophy of the hepatocytes in some of the males at the 50 mg/kg/day dose rate, but no changes were seen to the livers at any other dose. In the kidney there was a decrease in eosinophilic bodies in males at the highest dose; at 2 and 10 mg/kg there were no significant effects to the kidneys. Although not a statistically significant effect, it was noted that 2 males in the high dose group had dark kidneys while one male in the same group had an enlarged thyroid. There were no significant histopathologic changes to the spleen, heart, lungs or reproductive organs in males or females at the highest dose. At the dose of 50 mg/kg, there was a significant decrease in male and female body weight in the F1 generation. In a preliminary dose ranging study, male rats exposed to 250 mg/kg, in addition to the liver effects already described, showed an increase in histopathological effects to the kidneys (including regenerated basophilic tubules, dilatation of tubules, and necrosis of tubule epithelium). For the subchronic exposure of the F0 generation, the non-reproductive NOAEL was determined to be 10 mg/kg/day; similarly for the chronic exposure of the F1 generation, the non-reproductive NOAEL would be 10 mg/kg/day (*ibid*).

In a multigeneration reproduction study involving Sprague-Dawley rats exposed to 200, 650, and 2,000 ppm NP in the diet, effects were seen to the kidneys (an increase in renal medullary tubular dilation and cyst formation) in males in all generations (F0-F3) and in F3 females at lowest dose tested of 200 ppm (8-19 mg/kg/day in F0 males (average of 12 mg/kg/day); 9-35 mg/kg/day for males, in F1-F3 generations, 9-40 mg/kg/day in females in F1-F3 generations) (Chapin et al 1999). The authors conclude that NP exposure consistently produced adult kidney effects. It should be noted however that the same compound-related kidney effects were not observed in the subchronic study (Cunny et al 1997) in the same strain of rats administered the same dose levels of NP in the diet even though the duration of exposure in Cunny et al 1997 was similar to that for the F0 generation in the Chapin et al multigeneration study (90 days in Cunny et al 1997 and 105 days in Chapin et al 1999). In addition, the multigeneration study by Nagao et al, 2001 did not find any kidney effects at similar doses (the mid-range dose in Nagao et al is 10 mg/kg/day) as used in Chapin et al 1999. As stated in Environment Canada 2001a:

> "The renal lesions identified in the [Chapin et al] multigeneration study were described as being of minimal to mild severity, even at the higher dose levels, and were interpreted by the authors as a slight acceleration of the tubular nephropathy normally seen in this strain of rats (Chapin et al 1999). There was also no effect on serum urea nitrogen or creatinine at this dose in the subchronic study (Cunny et al 1997), suggesting that renal function was not affected (though urinalysis was not conducted in any study, and plasma urea concentration is not a sensitive marker of nephropathy). Based on these considerations, it seems likely that the LOEL of 12 mg/kg-bw per day is close to a No-Observed-Adverse-Effect-Level (NOAEL) for effects on the kidney..." (page 72).

The decision by Environment Canada 2001a to utilize the 12 mg/kg/day figure as a NOAEL is further reinforced by the results of Nagao et al 2001 (previously discussed) and a recent study by Latendresse et al 2001, in which kidney effects (polycystic kidney disease) were seen in Sprague Dawley rats fed NP at doses at or above 1,000 ppm in soy-free feed. Latendresse et al determined a NOAEL for this kidney effect at 500 ppm, which is similar to what was determined in Cunny et al 1997 (a NOEL of 650 ppm based on kidney effects). An interesting side note to Latendresse et al 2001 is that it appeared that the soy-free diet exacerbated the kidney effects, and the authors surmise that soy in the diet could act to ameliorate these effects.

3.1.4. Reproductive and Teratogenic Effects

NP and NPE have been determined to be weakly estrogenic in both *in vitro* and *in vivo* tests involving aquatic and terrestrial organisms (Blom et al 1998; Colborn et al 1993; Cox 1996; Environment Canada 2001a; Moffat et al 2001; Odum et al 1999a, b; Routledge and Sumpter 1996; Servos 1999; US EPA 1996; White et al 1994). In comparison to the natural estrogen 17-beta-estradiol, NP is approximately 1000 - 100,000 times weaker in eliciting estrogenic responses (Environment Canada 2001a; Giesy et al 2000; Moffat et al 2001; Muller and Schlatter 1998; Routledge and Sumpter 1996; Servos 1999; Sohoni and Sumpter 1998; US EPA 1996; White 1994). NP9E has also been found to be weakly estrogenic through *in vitro* tests, but is less potent than NP, by 1 to 3 orders of magnitude (Servos 1999; Sohoni and Sumpter 1998; US EPA 1996). In MCF-7 human breast cancer cells, cell proliferation was stimulated with OP, NP, NP2E, NP1EC[7] indicating estrogenicity (Blom et al 1998; White et al 1994). Using the

[7] NP1EC is nonylphenol ether carboxylate (also known as nonylphenoxy acetic acid) an environmental metabolite of NP9E.

recombinant estrogen yeast screen assay, Routledge and Sumpter 1996 showed that NP12E exhibited no estrogenic effect, while NP and NP2E showed dose-dependent elevations in estrogenic effect, although NP2E was approximately 500,000 times less potent than estradiol. X-77® and Activate Plus® (NPE-based surfactants) both induced MCF-7 proliferation at rates of 0.01 to 1 µg/ml, significantly higher than controls, indicating an estrogenic response. Both surfactants are 3 orders of magnitude less sensitive than estradiol or DES (Lin, Garry 2000). The data suggests that NPEs with >3 ethoxylate groups have little if any estrogenic effect (Servos 1999; US EPA 1996; White et al 1994). In general, estrogenic effects appear to decrease with increasing ethoxylate number.

NP increased uterine weight in immature or ovariectomized rats and in mice following oral administration of 75 mg/kg/day and above and following subcutaneous and intraperitoneal administration, with a NOAEL of 37.5 mg/kg/day (Environment Canada 2001a; Odum et al 1999b). Laws et al (2000) found this effect at 50 mg/kg, with a NOAEL of 25 mg/kg in immature rats and a LOAEL of 100 mg/kg and NOAEL of 50 mg/kg in adult ovariectomized rats. No evidence of estrogenic activity was observed in rats *in vivo* as evidenced by a lack of the stimulation of uterine growth following oral exposure of ovariectomized females to NP4E or NP9E at doses up to 1000 mg/kg/day for 3 or 4 days (Mitchell and Berke, 1995; Williams et al 1996, as cited in Environment Canada 2001a). *In vivo* tests in mammals have shown that high chronic dietary levels of NPE need to be administered to show any estrogenic effects (on the order of hundreds or thousands of ppm) (Cunny et al 1997; US EPA 1996).

Because of the demonstrated estrogenicity of NP, there have been many studies completed concerning potential reproductive effects of exposure. Many of the mammalian studies are described in Appendix 3, Table 1. Several are discussed below. There are relatively few reproductive tests completed concerning NP9E or other NPEs.

In a multigeneration reproduction study in rats, a 200-ppm daily dose of NP (the lowest dose tested) in the diet (12-18 mg/kg/day in males; 16-21 mg/kg/day in non-lactating females, 27-30 mg/kg/day in lactating females) was the LOEL based on kidney effects (Chapin et al 1999). No developmental effects were seen at any exposure level, however a range of effects on endocrine-regulated endpoints was observed at 650 and 2,000 ppm in females (increased estrous cycle length, accelerated vaginal opening, increase in relative weights of uterus and vagina). There were no consistent detectable effects on male reproductive parameters (*ibid*). A reproductive NOEL of 200 ppm (~12-40 mg/kg/day) was determined. The authors conclude that NP at low doses would appear to pose a greater hazard to the kidneys than to the reproductive system of male or female rats (*ibid*).

In a multigeneration study in rats where they were continuously exposed to NP via oral gavage at doses of 0, 2, 10, and 50 mg/kg/day, the authors concluded that the reproductive NOAEL for all three generations would be 10 mg/kg/day (Nagao et al, 2001). In this study, the F0 generation (6 week-old males and 13 week old females at the beginning of the test) showed no dose-related reproductive effects after exposure to NP at any dose. However effects were seen at the 50 mg/kg/day dose in the F1 generation. Although there were no treatment related effects on mating ability or fertility, there were effects to hormone levels in the F1 males and females at the highest dose, although the authors caution against assuming this is treatment related due to inconsistent changes in various related hormones and an absence of effect to the thyroid. There was also a significant decrease in both absolute and relative

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Refer to section 3.2.3.3.

ovary weight and an acceleration of vaginal opening. There was a significant decrease in the number of implants and live pups born to F1 females in the highest dose group. Histopathological examination found no treatment related effects to the testes, and spermatogenesis was normal; there was no effect on male fertility in any generation at any dose, which agrees with the findings in Chapin et al 1999.

De Jager et al 1999 provided oral doses of NP (0, 100, 250, and 400 mg/kg/day) to female rats during gestation through weaning (third week after birth) and to the male offspring from point of weaning through mating (10 weeks of age) (0, 100, 250 mg/kg/day) to determine both maternal effects and effects to male reproduction. There were no offspring born to the highest dose group. There were decreases in body weight and absolute testicular mass as well as structural effects to the male reproductive organs (decreased seminiferous tubule and lumen diameter, and a decrease in seminiferous epithelium thickness) at both 100 and 250 mg/kg/day dose levels (LOEL < 100 mg/kg/day). There were no significant effects to sperm count, or relative testis or epididymal weight at 100 mg/kg/day. In Nagao et al 2000, after subcutaneous injection of 500 mg/kg/day on post-natal days 1-5, rats were evaluated for reproductive function after puberty. There were effects to reproductive function in females, assumed to be the result of effects to the estrous cycle and histopathological alterations to the ovaries and uterus. In males, there was a decrease in germ cells in the seminiferous tubules, and an increase in degenerated germ cells was noted in the epididymides (*ibid*). There were no effects to sperm motility or plasma testosterone (*ibid*).

In a study using NP, interperitoneal exposure at 8 mg/kg/day during post-natal days 1-10 in male rats showed no effects to survival and no effects to male reproductive parameters (testis descent, organ weights). There were also no effects to kidney or liver relative weights (Odum, Ashby 2000). In an earlier study, Lee had come up with differing results using interperitoneal exposure to NP of neonatal male rat pups at 0, 0.08, 0.8, and 8 mg/kg/day from days 1 to 15 after birth that were sacrificed at age 31 days. In this experiment, the NOAEL for male reproductive effects (testis, epididymis, seminal vesicle, and prostate gland weights) was 0.08 mg/kg/day (Lee 1998). Liver weights were not affected at any dose; body weight was only affected at highest dose. Odum and Ashby could not duplicate the effects seen in Lee, 1998. They criticized the design used by Lee as leading to poor conclusions (Odum, Ashby, 2000).

NP9E was injected (intraperitoneal) into 9-10 week old male mice at doses of 20, 40, 50, 60 mg/kg/day for 5 days along with a positive and negative control to study the effects on sperm (Buttar et al, 1986). Evaluations were completed 35 days after injections were completed. The percentage of abnormal sperm was significantly different in the positive control group as compared to the treatment or vehicle control groups. The authors concluded that NP9E did not increase the frequency of morphologically abnormal sperm (NOAEL > 60 mg/kg.day).

No reproductive or developmental effects were observed following oral exposure during gestation to 600 mg/kg/day NP10E in mice (Hardin et al, 1987). NP10E was administered subcutaneously to 7-week old female rats at dose levels of 2 and 20 mg/kg/day for 15 weeks (Aso et al 1999a). There were no effects to reproductive ability and no effects to fetuses (external, skeletal or visceral effects). The same authors conducted another study in which NP10E was administered subcutaneously to female rats at dose levels of 5, 20, and 80 mg/kg/day from date of offspring birth through day 21 after birth to explore the effects on offspring from lactation exposure (Aso et al 1999b). There were no effects to physical development or reproductive ability, however there were growth effects at the highest dose. The authors consider 20 mg/kg/day to be the NOEL, based on growth effects to both the dams and offspring.

Oral exposure in rats to NP9E on gestation days 6-15 indicated a teratogenic NOEL at 50 mg/kg/day based on litter size decrease, pre-implantation loss, and skeletal anomalies seen in fetuses after maternal exposures to 250 and 500 mg/kg/day (Meyer et al 1988). These doses of 250 and 500 mg/kg/day were also maternally toxic, based on decreases in maternal weight gain. One study using intravaginal doses (single, 25 mg/kg) of NP9E in rats caused fetal resorptions, but was not teratogenic (Buttar, 1982b, abstract). Although the amount of absorption of NP9E from the vagina into the bloodstream has not been consistent (Buttar, 1982a; Malyk 1984), the ratio of NP9E and metabolites in the bloodstream as compared to the placenta and the fetus has shown a low rate of transfer (Buttar, 1982a). Another study using intravaginal doses of NP9E (4 or 40 mg/kg/day during gestation days 6-15) showed no embryotoxic or teratogenic effects in rats at dosages up to 25 times the clinical dose (Malyk, 1984).

The relationship between birth defects and use of NP9E or OP9E as spermicides was examined in an epidemiological study involving 462 women (426 of whom had used spermicides containing NP9E or OP9E in the first four months of pregnancy). Limb reduction deformities, neoplasms, Down's syndrome, and hypospadias did not occur in excess in children whose mothers were exposed to spermicides (Shapiro et al 1982). Although this provides no quantitative information, it is useful in that it is a study involving human health.

3.1.5. Carcinogenicity and Mutagenicity

NP9E was not mutagenic in the Ames test (either with or without metabolic activation) (Malyk, 1984; Meyer et al 1988; Shibuya et al, 1985). NP9E was not mutagenic based on the unscheduled DNA synthesis assay (adult rat hepatocytes) (Buttar et al 1986). NP9E did not induce malignant transformations (*in vitro*) in rat liver T51B cells (*ibid*). In one study, NP9E did induce malignant transformations in BALB/3T3 cells (Long et al 1982), however in another study using the same system, NP9E failed to induce transformations in BALB/3T3 cells (Sheu et al, 1988). In Sheu et al, 1988, the authors suggest that the differences between their results and Long et al 1982 could be due to impurities in the NP9E used in Long et al 1982. Analysis of the impurity 1,4-dioxane showed inducement of malignant transformations (Sheu et al 1988). NP10E did not induce proliferation of rat peritoneal cells using the Nashed method of rat peritoneal cell short-term carcinogenic test (Jinxi et al, 1992). NP10E was not mutagenic in the Ames test (either with or without activation) (Meyer et al, 1988).

In single studies, intraperitoneal exposure to NP9E did not induce cell proliferation in peritoneal cells in rats or abnormalities in germ cells in mice (Buttar et al 1986; Jinxi et al 1992). In one study, exposure to X-77® (NP9E-based) surfactant showed dose dependent positive results in an assay of genotoxicity (Garry et al 1999). NP4E showed no evidence of genotoxicity in tests of reverse mutation in bacteria or in unscheduled DNA repair studies in rat primary hepatocytes. NP4E did not induce micronuclei in the bone marrow cells of mice following intraperitoneal injection in one study (WHO, 1998 as cited in Environment Canada 2001a).

NP did not show any initiating activity for BALB/3T3 cell transformation, implying that NP did not cause any genetic alteration that was inherited by daughter cells (Sakai 2001). In another study, NP did cause transformation of pre-treated BALB/3T3 cells in the promotion phase, but not in the initiation phase, indicating that NP may cause the enhancement of carcinogenesis *in vivo* (Sakai 2001). NP was consistently negative in bacterial tests of mutagenicity, although it induced DNA breaks in human sperm, lymphocytes, and MCF-7 breast cancer cells exposed *in vitro* (WHO, 1998; Banerjee and Roy, 1996 (abstract); Anderson et al., 1997). NP did induce mammary gland cell proliferation in a study on female Noble rats (Colerangle and Roy, 1996), but the method of exposure (subcutaneous pumps) and

the inability of other researchers to duplicate these findings put the relevance of this study by Colerangle and Roy into question.

No evidence of carcinogenicity was reported in 2-year chronic oral toxicity studies of NP9E with rats and dogs (Smyth and Calandra, 1969; refer also to section 3.1.3.1 above). Intravaginal dosages of NP9E in rats, up to 20 times the rates recommended for use in humans as a spermicide, for 2 years, indicated no carcinogenicity (unpublished Ortho Pharmaceutical study discussed in Malyk, 1984).

No chronic toxicity studies with NP were found with the exception of the two multigeneration studies discussed above (Chapin et al 1999; Nagao et al 2001). There was no indication of carcinogenesis in either of these studies.

As stated in European Union 2002:

> "Carcinogenicity has not been directly studied. However, some information on the carcinogenic potential can be derived from other data. On the basis of information currently available it is unlikely that nonylphenol is mutagenic, so concerns for cancer caused by a genotoxic mechanism are low. Considering the potential for carcinogenicity by a non-genotoxic mechanism, no evidence of sustained cell proliferation or hyperplasia was seen in the standard repeated exposure toxicity studies. Nonylphenol has been reported to induce cell proliferation in the mammary gland of the Noble rat following subcutaneous exposure levels down to 0.05 mg/kg/day, but this finding could not be reproduced in a duplicated study; furthermore there are doubts about the relevance of this model to humans because of the route of exposure and sensitivity of the strain selected. Overall, there are low concerns for carcinogenicity by a non-genotoxic mechanism." (page 19)

3.1.6 Effects on the Skin and Eyes

Acute dermal LD_{50} of NP9E in rabbits is greater than 2,830 mg/kg. NP9E is considered minimally to severely irritating to rabbit skin and moderately to severely irritating to rabbit eyes (WHO, 1998, as cited in Environment Canada 2001a; Smyth and Calandra, 1969). Acute dermal LD_{50} of NP5E in rabbits is greater than 2,000 mg/kg; with NP6E in rabbits, the acute dermal LD_{50} exceeds 3,000 mg/kg (CTFA, 1979a, 1979b, unpublished). Both NP5E and NP6E are considered at most, slightly toxic to rabbits via dermal exposure. NP5E and NP6E are skin irritants in rabbits; NP6E is not a skin sensitizer in guinea pigs (Nethercott and Lawrence, 1984). The ocular irritation potential of NP6E was evaluated in a Draize test using rabbits; the eyes were not rinsed. NP6E was classified as a severe ocular irritant (Consumer Product Testing Company, 1978, unpublished). NP5E caused severe ocular reactions (species not noted) (CTFA, 1979a, unpublished). These values indicate that NPEs are in EPA toxicity category III or IV.

Dermal acute toxicity of NP in rabbits gives LD_{50} values > 2,000 mg/kg. NP is considered moderately to severely irritating to rabbit skin and eyes (WHO, 1998; U.S. EPA 1992a, b, c (unpublished reports) as cited in Environment Canada 2001a). This value indicates that NP is in EPA toxicity category III.

Contact dermatitis has been reported in humans following exposure to various NPEs in consumer products. Photosensitization was also seen in patients suspected of photodermatosis when exposed to NP10E (Michel et al, 1994). Meding presents a case study of one man with dermatitis after working with a fluid containing NPE (unspecified type). Patch testing with NPE indicated positive reaction. Dermatitis cleared after retirement, but relapsed on occasion after contact with detergents. Patch testing

of 165 comparison subjects with 1% NPE were negative in all cases (Meding 1985). Nethercott and Lawrence present a case study of dermatitis in a worker exposed to hand cleanser at work. The patient reacted to NP6E, but not to the other components of the cleanser, including OP9E. Patch testing with NP6E on control test subjects indicated no reactions at 0.5% concentration. There was no sensitization in a small test of guinea pigs exposed to NP6E; the authors conclude that the practical risk of sensitization must be extremely low (Nethercott, Lawrence 1984). Dooms-Gossens et al report on an instance of twelve cases of contact sensitivity to three antiseptic solutions. Testing using active ingredients and 2% solutions of the nonoxynol carriers (NP8.3E, NP9E, NP10E) indicated the reaction was caused by the nonoxynol carrier (Dooms-Gossens et al 1989). A follow-up on six of the twelve patients indicated varying sensitivities to NP6, 8.3, 9, 10, 14, 18E, while 20 tested controls showed no sensitivity (*ibid*). Wilkinson et al report on a case study of allergic contact dermatitis in a 45-year old female exposed to a furniture polish at work. She was patch-tested with the individual ingredients of the polish and reacted to all, including NP12E, while 30 controls showed no reaction (Wilkinson et al 1995).

In three unpublished studies cited in Johnson 1999 (Jordan 1994, 1995a, 1995b), 5% and 10% NP2E and 10% NP4E were evaluated as skin sensitizers on humans. There was no evidence of allergic contact dermatitis when re-exposed for 48 or 96 hours to 5% NP2E (Jordan 1994). Sensitization was seen with NP2E at 10% dilution, with 9 of 103 subjects developing contact allergic dermatitis (Jordan 1995a). Seven of these nine were retested using a 30-minute exposure; after 24 hours only 2 showed a mild allergic response. Sensitization was seen also with NP4E at 10% dilution, with 3 of 107 subjects developing contact allergic dermatitis. These three were retested with a 30-minute exposure; after 24 hours only 1 showed a mild allergic response (Jordan 1995b).

3.1.7. Systemic Toxic Effects from Dermal Exposure

In one study on rats, NP9E was administered dermally to females during gestational days 6-15 at doses of 0, 50, or 500 mg/kg/day. There were no dose-related reproductive or teratogenic effects following this dermal exposure, although there was a decrease in feeding in dams exposed to the highest dose (Meyer et al 1988).

It has been shown that NP9E applied dermally can increase water absorption through the skin (An-Ex Analytical Services Ltd., 1995a, unpublished). Skin absorption of NP2E, NP4E, and NP9E through *in vitro* tests on human female skin showed no quantifiable absorption after 1-hour or 48-hour exposure (An-ex Analytical Services Ltd, 1995c, unpublished). Based on the limits of detection of the methods used, the estimated absorption was <0.19% for NP2E, <1.04% for NP4E, <1.33% for NP9E, but these are thought to be high figures (*ibid*). These values can be converted to an hourly rate by dividing by 48, resulting in <0.00004 hr^{-1}, <0.0002 hr^{-1}, and <0.0003 hr^{-1} respectively. In another study, after a 48-hour exposure, these same materials showed 0.57% for NP2E, 0.66% for NP4E and 0.49% for NP9E of applied dose absorbed (An-ex Analytical Services Ltd, 1995e; Clairol, Inc. 1995). Converting these values to an hourly rate results in 0.0001 hr^{-1} for all three compounds. In a published study (Monteiro-Riviere et al, 2000), 8-hour *in vitro* exposures to NP, NP4E, and NP9E were performed on rat, pig, and human skin. This study found that 1% NP9E in water or PEG-400 resulted in absorption into all three species in the range of 0.05 to 0.06% of applied, which would work out to 0.00006 to 0.00008 $hour^{-1}$ (*ibid*). Absorption of NP4E and NP were similar to NP9E (*ibid*). These values are in line with the values calculated in the Worksheets in Appendix 4 (Worksheet B05 summarizes both first order and zero-order absorption rates). Calculated values for zero-order absorption are 0.0001 (0.000039 – 0.00028) cm/hour; and for first-order absorption the calculated rates are 0.00007 (0.00001 to 0.0009)

hour^{-1}. Calculation of the first order and zero-order absorption rates as displayed in Worksheets A07a and b and B03 and B04 in Appendix 4 uses the methods outlined in SERA 2000.

Based on studies on eight pesticides with molecular weights ranging from 221 to 416 and log K_{ow} values ranging from -0.75 to 6.50, SERA 2000 developed estimates of exposure rates in workers and these estimates are used in this risk assessment. Although the molecular weight of NP9E is outside this range, and NP9E is a mixture rather than a single compound, the values derived in SERA 2000 should be conservative for this use, in as much as larger molecules would tend to be absorbed at lower rates. The results from the An-ex Analytical Services Ltd studies indicate a lack of much variability in absorption of shorter NPEs as compared to NP9E, indicating that the fact that NP9E is a mixture of various length NPEs should not affect the calculation of absorption figures.

The absorption figures in the An-ex Analytical Services Ltd studies were criticized in Environment Canada 2001a as coming from studies lacking the use of viable skin, not analyzing for NP or NPEs or metabolites in the skin or metabolites in the receptor fluid, and not describing temporal changes in flux across the skin. IN APERC 2000, an unpublished study is described that shows that at most, 1% of the applied dose of NP, NP4E, or NP9E is absorbed through human and porcine skin. It is unclear whether this unpublished study deals with the criticisms that are presented in Environment Canada 2001a.

3.1.8. Immunotoxicity

Some xenoestrogenic chemicals may also have an effect on the immune system; estradiol and diethylstilbestrol have shown both types of effects (Ahmed 2000; US EPA 1997). Octylphenol has been found to affect the thymus (Ahmed 2000). In one study using female mice (Caren and Brunmeier, 1987) the mice were injected with 0.2 ml of 0.2% NP9E daily (approximately 130 mg/kg/day) for 24 days followed by a challenge with sheep red blood cells. There were no effects to leucocyte counts, primary and secondary anti-SRBC titers, and serum immunoglobulin M (IgM) and serum immunoglobulin G (IgG) concentrations. There was a decrease in body weight gain and relative liver weight, and an increase in relative spleen weight compared to controls (*ibid*). There were no changes noted to the heart, kidneys, lungs, or thymus.

In an *in vitro* study using male mice spleen cells, exposure to NP affected proliferation of the cells (Yamashita, et al, 2002). Using the lymphocyte proliferation assay, NP was found to enhance proliferative responses of spleen cells, and enhanced the production of cytokines, such as interleukin, interferon, and total immunoglobulin production (*ibid*). This could indicate a risk of hypersensitivity or autoimmunity.

Indirect observations of potential immunotoxicity can be developed from *in vivo* studies that conduct histopathological examinations of body tissues that are part of the immune system such as the lymphoid tissues (lymphocytes), thymus, spleen, bone marrow, and lymph nodes (SERA 2002). In Nagao et al, 2001, after continuous exposure to NP (oral gavage) at 50 mg/kg/day in rats, there was a decrease in both relative and absolute thymus weight, but no histopathologic alterations observed in this organ; these effects were not seen at the next lower dose of 10 mg/kg. In the same study, after exposure of males to 250 mg/kg/day over several months, reduced thymus was observed in most of the males, and upon histopathologic examination, there was atrophy with pyknosis (reduction in the nucleus) and a reduction in lymphocyte number. Based on this observation, it was felt that the reduced thymus weights seen at 50 mg/kg were likely related to the exposure to NP (*ibid*).

In the study by Cunny et al 1997 discussed in Section 3.1.3.2., there was no effect to spleen weight, and histopathological examinations of sternum bone marrow, the spleen, mandibular and mesenteric lymph nodes, and the thymus reveled no treatment related changes after a 90-day exposure to NP in male and female rats up to 129 (males) and 149 mg/kg/day (females). In the multigeneration study by Chapin et al 1999, there were no effects to the spleen, in terms of relative weight, in any generation at any NP dose tested (up to 2,000 ppm).

3.1.9 Neurotoxicity.

There are few studies that look at neurological effects of exposure to NP9E or the other NPEs. In Aso et al 1999a, after subcutaneous injection of NP10E in the female rats at 2 and 20 mg/kg/day for 15 weeks, effects to offspring that were conceived and delivered during the maternal exposure period showed no effects in several behavior tests (open field test, water maze test), nor showed any effects in several reflex response assessments (righting on surface, negative geotaxis, corneal or pinna reflex).

There is a study of exposure to NP2E in fish that indicates a potential for central nervous system interaction (Cravedi et al, 2001). In this study, rainbow trout were exposed to 10 mg/kg NP2E via oral gavage. Analysis of various tissues for radiolabeled NP2E indicated presence in the brain. The authors state that although residues in the brain were low, its presence indicates the ability of NP2E (or its metabolites) to penetrate the blood-brain barrier and could affect the central nervous system functions. The study by Cravedi et al did not examine whether any nervous system effects were caused by this exposure.

There are several *in vivo* studies that look at the neurological effects of exposure to NP. In a recent multigenerational study by Flynn et al, 2002, Sprague-Dawley rats were exposed to NP in the diet at rates of 0, 25, 200, 750 ppm (equivalent to 0, 2, 16, 60 mg/kg/day) over two generations (F_0, F_1). Females in each of three generations (F_0, F_1, F_2) were tested at several points during their lives using a Morris water maze test. The study showed that two generations of dietary exposure to NP did not significantly alter the Morris water maze performance in young adult or middle-aged female rats. This suggests that chronic dietary exposure to NP does not cause gross alterations in spatial learning and memory in female rats.

In a multigeneration NP exposure study in rats (Nagao et al, 2001) performance in behavioral tests (open field activity, water maze, and running wheel activity) was assessed, as was the development of neural reflexes (righting response, cliff-drop aversion response, negative geotaxis) in developing pups. There were no significant effects seen in any of these parameters in the F1 or F2 generations after lifetime exposures to up to 50 mg/kg/day NP via oral gavage. Although females in the F1 generation showed a significant delay in achievement of negative geotaxis compared to controls at the 50 mg/kg dose, the values were within the historical range of the lab so the authors did not consider it an NP-related effect. There was an increase in salivation in F0 males at 50 mg/kg.

In a study by Ferguson, et al 2000, pregnant Sprague-Dawley rats were exposed to NP in the diet at 0, 25, 500, and 2,000 ppm and after weaning, their offspring were exposed to the same diet until postnatal day 77. Although the data presented in the study does not allow for precise calculations of these feeding rates in terms of mg/kg/day, using several assumptions as to initial weight of female parents, the maternal dose is probably about 3-5, 40-60, and 150-200 mg/kg/day for the 25, 500, and 2,000 ppm diets. At several points during the growth of the offspring, behavioral tests were conducted to assess effects of NP exposure. There were no consistent NP-related effects in open-field activity, running

wheel activity, play behavior, or intake of a saccharin-flavored solution. Intake of a sodium-flavored solution as well as water intake was increased at the 2,000 ppm level in offspring. The authors note that increased sodium solution intake has been seen in experiments after developmental exposure to other estrogenic compounds (such as genistein and estradiol), indicating that this may be an estrogenic response. If this is a neurotoxic effect, the LOEL for such an effect may be around 150 mg/kg/day.

In a study by deJager et al 1999, male rats exposed to NP during development and weaning (through maternal dosing), and after weaning (oral gavage) showed no signs of behavioral abnormalities when exposed to NP up to 250 mg/kg/day through post natal day 70. The study does not describe how behavior was assessed.

Indirect observations of potential neurotoxicity can be developed from in vivo studies that conduct histopathological examinations of body tissues that are part of the nervous system such as the spinal cord, the brain, peripheral nerves (such as the sciatic nerve) (SERA 2002). In the study by Cunny et al, 1997 as discussed in Section 3.1.3.2., there were no effects seen to the brain or brainstem in terms of absolute weight or based upon microscopic examination of the tissues after subchronic 90-day exposures to NP up to 149 mg/kg/day in male or female rats. In a study to determine the *in vivo* metabolites of NP in rats, the lipophilic NP-aglycone metabolite was found in brain tissue after multigeneration exposure to 750 ppm of NP in feed (Doerge et al 2002).

An *in vitro* study looked at the effects of NP on a rat pheochromocytoma cell line PC12 (a cell line that responds to nerve growth factor) (Talorete et al, 2001). The study determined that exposures to NP up to 300 μM for 15 minutes had no effect on cell necrosis. There were no effects on DNA fragmentation (apoptosis) at levels of NP at 50, 100, and 500 μM for 4 hours. The authors concluded that apoptosis would not occur at levels that are believed to be estrogenic to estrogenic cell-lines, such as MCF-7 cells. However, the study did find inhibitory effects to nerve growth factor-induced acetylcholinesterase activity at levels as low as 0.8 μM, indicating a potential for neurological and behavioral problems, although the responses were not dose-related.

3.1.10 Impurities, Metabolites, and Inerts

Metabolites

The potential effects of metabolites on a risk assessment are often encompassed by the available *in vivo* toxicity studies under the assumption that the toxicologic consequences of metabolism in the species on which toxicity studies are available will be similar to those in the species of concern. Uncertainties in this assumption are encompassed by using uncertainty factors when analyzing for interspecies differences.

Based on one study of NP9E, it appears to be rapidly metabolized and excreted (Walter et al 1988). After injection of NP9E into female Sprague-Dawley rats, bile and urine were monitored for metabolites. The NP9E was completely metabolized by the rats and these metabolites were primarily excreted in feces and secondarily in urine (all radioactivity being excreted within 48 hours after injection). Analysis of urinary metabolites 24 hours following an i.v. dose indicated the presence of neutral and acidic species.

Doerge et al 2002 analyzed for NP metabolites in rats after feeding over 2 generations at levels of 1.5, 12, and 45 mg/kg/day. Glucuronides were identified as the primary metabolite, with lesser amounts of NP-aglycone and NP-catechol. Glucuronides are not active as an estrogen receptor (nor as anti-

estrogens, androgens, or anti-androgens) while the NP-aglycone and NP-catechol are expected to continue to act as estrogen mimics (*ibid*; Moffat et al, 2001; Madigou et al 2001). After a 50 mg/kg oral dose, there was rapid absorption and elimination of NP in both males and females (elimination halftimes of 3.1 to 4.0 hours) (*ibid*). In a human exposure experiment to NP, radio-labeled NP was injected intravenously (14 µg/kg) or given orally (66 µg/kg) to two human volunteers to study metabolism and excretion (Müller et al, 1998). Elimination from the blood was rapid, with no detectable residue after 10 hours through either method of exposure. Only a relatively small percentage of NP or glucuronide or sulphate conjugates were detectable in the urine or feces (approximately 10% of the dose), suggesting further metabolism to compounds unidentified in this study or storage in tissues, likely lipids (*ibid*). Analysis of adipose tissues from human cadavers indicated concentrations in the range of 19.8 to 84.4 ng NP/g lipid (*ibid*).

Metabolism of short-chain nonoxynols involves shortening of ethylene oxide chain and some carboxylation of the alkyl chain. No formation of free phenolic groups has been seen (Johnson 1999).

Tanghe et al 2000 showed that octylphenol degraded to a metabolite that involved the intact octyl chain as a tertiary alcohol. The authors assumed that bacteria utilize the carbons in the phenolic ring, leaving the alkyl chain intact. According to the authors, as a result of the biotransformation of octylphenol, its estrogenic potency was removed because it is the phenolic moiety that interacts with the estrogen receptors.

Impurities

To some extent, concern for impurities in technical grade NPE is reduced by the fact that the existing toxicity studies on NPE were conducted with the technical grade product. Thus, if toxic impurities are present in the technical grade product, they are likely to be encompassed by the available toxicity studies on the technical grade product. An exception to this general rule involves carcinogens, most of which are presumed to act by non-threshold mechanisms. Because of the non-threshold assumption, any amount of a carcinogen in an otherwise non-carcinogenic mixture may pose a carcinogenic risk. This is the situation with NPE. NPE may contain ethylene oxide and 1,4-dioxane as impurities.

Ethylene oxide has been found in NP9E at low levels, <3.6 to 12.2 ppm, in the unreacted form as a residual from the manufacturing process (Johnson 1999). Depending upon processing methods, this can be reduced essentially to zero. Ethylene oxide is used in the production of many chemicals, including ethoxylates, and used as a hospital sterilant, but most use is for the production of ethylene glycol. Ethylene oxide is likely present in many products that contain ethoxylates, such as surfactants containing linear alcohol ethoxylates. Unreacted levels of ethylene oxide in these products should reduce with time due to reaction, storage, further pumping, and other processing.

Ethylene oxide has been described as a probable human carcinogen with sufficient evidence in experimental animals to support a finding as a carcinogen; it is also a mutagen (refer to Appendix 2). Ethylene oxide has a high vapor pressure and high water solubility, and at normal room temperature and pressure is a gas. Because of its high vapor pressure and high water solubility it is not expected to bioaccumulate or accumulate in soil or sediment (Environment Canada 2001b). Metabolism of ethylene oxide in larger mammals is primarily through hydrolysis to ethylene glycol, which in turn is converted to oxalic acid, formic acid, and CO_2. (*ibid*). While a detailed review of ethylene oxide is beyond the scope of this risk assessment, adequate information is available on ethylene oxide to quantify the carcinogenic risk associated with the use of NP9E. This discussion of risk is contained in Appendix 2.

Based on conservative assumptions concerning exposure, the carcinogenic risks to workers from ethylene oxide are at acceptable levels (Appendix 2). Ethylene oxide will not be discussed further in this risk assessment.

1,4-dioxane has also been found as an impurity in NP9E at low levels (<4.5 to 5.9 ppm) (Johnson 1999). 1,4-dioxane has also been classified as a carcinogen. Borrecco and Neisess 1991 conducted a risk assessment of the impurity 1,4-dioxane in the surfactant in Roundup formulations of glyphosate. In that risk assessment, they assumed a concentration of 1,4-dioxane at 0.03% in the Roundup formulation, which is about two orders of magnitude greater concentration than found in NP9E as described in Johnson, 1999. Borrecco and Neisess used a systemic NOEL of 9.6 mg/kg/day and a cancer potency value of 0.0076 mg/kg/day. With the higher percentage of 1,4-dioxane assumed in Roundup, they concluded that the risk of acute, chronic, or reproductive effects would be acceptably low, even at maximum labeled rates for Roundup. They included a cancer risk assessment written by Heydens 1989, which looked at the increased risk of cancer caused by the use of surfactants that contained 1,4-dioxane as a contaminant. In this risk assessment, Heydens using a cancer potency value of 0.0076 mg/kg/day, and a 300 ppm contamination rate, determined that the risk of cancer from 1,4-dioxane was well below the 1 in 1 million threshold considered acceptable. Heydens concluded that the carcinogenic risk from exposure to 1,4-dioxane is negligible for occupationally exposed individuals. As these two documents have adequately considered the risk of 1,4-dioxane, this impurity will not be considered further in this risk assessment.

It is important to note that chronic studies involving NP9E have not determined cancer to be an endpoint in mammals (section 3.1.5).

Inerts

Most commercial NPE-based surfactants contain an alcohol such as butanol or isopropanol, as well as a silicone defoamer, and water. All of these items are on U.S. EPA inerts list 4A or 4B, and are therefore considered by U.S. EPA to be safe for use in pesticide formulations. There is no reason to assert that these compounds will materially impact the risks associated with the use of NPE-based surfactants.

3.2 Exposure Assessment

3.2.1. Overview

There are no occupational exposure studies in the available literature that are associated with the application of NP9E in a surfactant formulation. Consequently, worker exposure rates are estimated from an empirical relationship between absorbed dose per kilogram of body weight and the amount of chemical handled in worker exposure studies on nine different pesticides (SERA 1998).

For ground-based, backpack applications, central estimates of worker exposure are 0.01 mg/kg/day. The upper limits of the exposure estimates are 0.53 mg/kg/day. For the boom sprayer, central estimates of worker exposure are 0.037 mg/kg/day, with an upper limit of 1.01 mg/kg/day. Aerial applications result in potential exposure rates that are intermediate between these backpack and boom applications.

Except in the case of accidental exposures, the levels of NP9E to which the general public might be exposed should be far less than the levels for workers. Longer-term exposure scenarios for the general public lead to central estimates of daily doses in the range of about 0.000001 to 0.00032 mg/kg/day with upper limits of exposure in the range of 0.000002 to 0.020 mg/kg/day. While these exposure scenarios

are intended to be conservative, they are nonetheless plausible. Accidental exposure scenarios result in central estimates of exposure of up to 0.46 mg/kg/day with upper ranges of 1.71 mg/kg/day. All of the accidental exposure scenarios involve relatively brief periods of exposure, and most should be regarded as extreme, some to the extent of limited plausibility.

3.2.2 Workers

A summary of the estimated exposures to NPE involving workers is presented in Table 3-1. Two types of exposure assessments are considered: general and accidental/incidental. The term general exposure assessment is used to designate those exposures that involve estimates of absorbed dose based on the handling of a specified amount of a chemical during specific types of applications. The accidental/incidental exposure scenarios involve specific types of events that could occur during any type of application. Details regarding all of these exposure assessments are presented in the worksheets that accompany this risk assessment.

3.2.2.1. General Exposures

As described in SERA 2000, the ranges of estimated occupational exposure rates vary substantially among individuals and groups, (i.e., by a factor of 50 for backpack applicators and a factor of 100 for mechanical ground sprayers). It seems that much of the variability can be attributed to the hygienic measures taken by individual workers (i.e., how careful the workers are to avoid unnecessary exposure).

The estimated number of acres treated per hour is taken from previous Forest Service risk assessments, except for backpack applications, which uses a Pacific Southwest Region average of 2 acres per day per worker. The number of hours worked per day is expressed as a range, the lower end of which, 6 hours per day, is based on an 8-hour work day with 1 hour at each end of the work day spent in activities that do not involve herbicide exposure. The upper end of the range, 8 hours per day, is based on an extended (10-hour) work day, allowing for 1 hour at each end of the work day to be spent in activities that do not involve herbicide exposure. It is recognized that the use of 6 hours as the lower range of time spent per day applying herbicides is not a true lower limit. It is conceivable and perhaps common for workers to spend much less time in the actual application of a herbicide if they are engaged in other activities. Thus, using 6 hours can be regarded as conservative. In the absence of any published or otherwise documented work practice statistics to support the use of a lower limit, this conservative approach is used.

The range of acres treated per hour and hours worked per day is used to calculate a range for the number of acres treated per day. For this calculation as well as others in this section involving the multiplication of ranges, the lower end of the resulting range is the product of the lower end of one range and the lower end of the other range. Similarly, the upper end of the resulting range is the product of the upper end of one range and the upper end of the other range. This approach is taken to encompass as broadly as possible the range of potential exposures.

The range of application rates and the typical application rate are taken directly from the program description (see section 2.4). The typical estimate of 0.020 gallons NP9E/acre (1.67 lbs/acre) is based upon Forest Service experience using a common herbicide, glyphosate. The upper end of the range of application rates is taken as 0.8 gallons/acre a.i./acre (6.68 lbs/acre), one of the higher recommended label rates. The lower limit of the application rate is taken as 0.020 gallons a.i./acre (0.167 lbs/acre), the lowest recommended label rate commonly seen.

The typical, or central, estimate of the amount handled per day is calculated as the product of the central

estimate of the acres treated per day and the typical application rate. The ranges for the amounts handled per day are calculated as the product of the range of acres treated per day and the range of application rates. Similarly, the central estimate of the daily absorbed dose is calculated as the product of the central estimate of the exposure rate and the central estimate of the amount handled per day. The ranges of the daily absorbed dose are calculated as the product of the range of exposure rates and the range for the amounts handled per day.

3.2.2.2. Accidental Exposures

Typical occupational exposures may involve multiple routes of exposure (i.e., oral, dermal, and inhalation); nonetheless, dermal exposure is generally the predominant route for herbicide applicators. Typical multi-route exposures are encompassed by the methods used in section 3.2.2.1 on general exposures. Accidental exposures, on the other hand, are most likely to involve splashing a solution of surfactant into the eyes or a variety of dermal exposure scenarios.

NP9E and NP can cause irritant effects in the eyes (see section 3.1.6). The available literature does not include quantitative methods for characterizing exposure or responses associated with splashing a solution of a chemical into the eyes; furthermore, reasonable approaches to modeling this type of exposure scenario quantitatively are not apparent. Consequently, accidental exposure scenarios of this type are considered qualitatively in the risk characterization (section 3.4).

There are various methods for estimating absorbed doses associated with accidental dermal exposure. Two general types of exposure are modeled: those involving direct contact with a solution of the surfactant and those associated with accidental spills of the surfactant onto the surface of the skin. Any number of specific exposure scenarios could be developed for direct contact or accidental spills by varying the amount or concentration of the chemical on or in contact with the surface of the skin and by varying the surface area of the skin that is contaminated.

For this risk assessment, two exposure scenarios are developed for each of the two types of dermal exposure and the estimated absorbed dose for each scenario is expressed in units of mg chemical/kg body weight. Details of these exposure estimates are presented in the worksheets appended to this risk assessment.

Exposure scenarios involving direct contact with solutions of the chemical are characterized by immersion of the hands for 1 minute and wearing contaminated gloves for 1 hour. Generally, it is not reasonable to assume or postulate that the hands or any other part of a worker will be immersed in a solution of a surfactant for any period of time. On the other hand, contamination of gloves or other clothing is quite plausible. For these exposure scenarios, the key element is the assumption that wearing gloves grossly contaminated with a chemical solution is equivalent to immersing the hands in a solution. In either case, the concentration of the chemical in solution that is in contact with the surface of the skin and the resulting dermal absorption rate are essentially constant.

For both scenarios (the hand immersion and the contaminated glove), the assumption of zero-order absorption kinetics is appropriate. Following the general recommendations of U.S. EPA, Fick's first law is used to estimate dermal exposure.

Exposure scenarios involving chemical spills on to the skin are characterized by a spill on to the lower legs as well as a spill on to the hands. In these scenarios, it is assumed that a solution of the chemical is spilled on to a given surface area of skin and that a certain amount of the chemical adheres to the skin. The absorbed dose is then calculated as the product of the amount of the chemical on the surface of the skin (i.e., the amount of liquid per unit surface area multiplied by the surface area of the skin over which the spill occurs and the concentration of the chemical in the liquid), the first-order absorption rate, and the duration of exposure. For both scenarios, it is assumed that the contaminated skin is effectively cleaned after 1 hour. As with the exposure assessments based on Fick's first law, this product (mg of absorbed dose) is divided by body weight (kg) to yield an estimated dose in units of mg chemical/kg body weight.

Table 3-1: NPE - Summary of Worker Exposure Scenarios

Scenario	Dose (mg/kg/day or event) Typical	Lower	Upper
General Exposures			
Directed ground spray (backpack)	0.01	0.000075	0.53
Boom sprayer	0.037	0.00011	1.01
Aerial Application	0.024	0.00004	0.53
Accidental/Incidental Exposures			
Immersion of Hands, 1 minute	0.00017	0.000031	0.0011
Contaminated Gloves, 1 hour	0.010	0.0019	0.066
Spill on hands, 1 hour	0.000054	0.0000038	0.0017
Spill on lower legs, 1 hour	0.00013	0.0000095	0.0043

3.2.3 General Public

Under normal conditions, members of the general public should not be exposed to substantial levels of NPE surfactants or herbicides. Nonetheless, any number of exposure scenarios can be constructed for the general public, depending on various assumptions regarding application rates, dispersion, canopy interception, and human activity. Several highly conservative scenarios are developed for this risk assessment.

The two types of exposure scenarios developed for the general public includes acute exposure and longer-term or chronic exposure. All of the acute exposure scenarios are primarily accidental. They assume that an individual is exposed to the compound either during or shortly after its application. Specific scenarios are developed for direct spray, dermal contact with contaminated vegetation, as well as the consumption of contaminated fruit, water, and fish. Most of these scenarios should be regarded as extreme, some to the point of limited plausibility. The longer-term or chronic exposure scenarios parallel the acute exposure scenarios for the consumption of contaminated fruit, water, and fish but are based on estimated levels of exposure for longer periods after application.

The exposure scenarios developed for the general public are summarized in Table 3-2. As with the worker exposure scenarios, details of the assumptions and calculations involved in these exposure

assessments are given in the worksheets that accompany this risk assessment. The remainder of this section focuses on a qualitative description of the rationale for and quality of the data supporting each of the assessments.

3.2.3.1. – Direct Spray

Direct sprays involving ground applications are modeled in a manner similar to accidental spills for workers (see section 3.2.2.2.). In other words, it is assumed that the individual is sprayed with a solution containing the compound and that an amount of the compound remains on the skin and is absorbed by first-order kinetics. As with the similar worker exposure scenarios, the first-order absorption kinetics are estimated from the empirical relationship of first-order absorption rate coefficients to molecular weight and octanol-water partition coefficients.

For these exposure scenarios, it is assumed that during a ground application, a naked child is sprayed directly with NP9E. These scenarios also assume that the child is completely covered (that is, 100% of the surface area of the body is exposed). These are extremely conservative exposure scenarios and are likely to represent upper limits of plausible exposure. An additional set of scenarios are included involving a young woman who is accidentally sprayed over the feet and legs. For each of these scenarios, standard values are used regarding the surface area of the skin and body weight.

3.2.3.2. Dermal Exposure from Contaminated Vegetation

In this exposure scenario, it is assumed that the herbicide is sprayed at a given application rate and that an individual comes in contact with sprayed vegetation or other contaminated surfaces at some period after the spray operation. For these exposure scenarios, some estimates of dislodgeable residue and the rate of transfer from the contaminated vegetation to the surface of the skin must be available. No such data are directly available for NPE, and the estimation methods of Durkin et al. 1995 are used. Other estimates used in this exposure scenario involve estimates of body weight, skin surface area, and first-order dermal absorption rates, as discussed in the previous section.

3.2.3.3. - Contaminated Water

Water can be contaminated from runoff, as a result of leaching from contaminated soil, from a direct spill, or from unintentional contamination from applications. For this risk assessment, the three types of estimates made for the concentration of NPE in water are two accidental scenarios and one contamination level based on operational drift or movement through soil. The accidental scenarios are based on a either a spill of a fixed amount of NPE into a body of water of a fixed size assuming instantaneous mixing, or the overspray of a stream segment. The operational scenario is based on the assumption that the NP9E is being applied to water in proportion to the herbicide glyphosate, which is assumed to be present at the limit of detection (25 ppb) of current water monitoring efforts.

Research has shown that in the presence of oxygen (oxic or aerobic conditions), NP9E is biodegradable in soil or water, with a lab-tested half-life of a few to up to 30 days (APERC 1998; Maguire 1999; Naylor 1999; Staples et al 2001). In aerobic conditions, NP9E, and other NPEs, are broken down through the removal of ethoxylate groups as a result of microbial action or photolysis, into shorter-chain ethoxylates (John et al 2000; John, White 1998; Maki et al 1996; Castillo et al 2001; Manzano et al 1998). Some studies show this breakdown resulting in formation of short-chain NPE (NP1E or NP2E) (John et al 2000; Tanghe et al 1999, Castillo et al 2001), however most studies indicate that further reactions cause formation of short-chain nonylphenol ether carboxylates (NP1EC, NP2EC) (Ahel et al

1994a, 1994b; APERC 1999a; Di Corcia et al 1998; Jonkers et al 2001; Manzano et al 1998 and 1999; Maguire 1999; Maki et al 1996; US EPA 1996). These shorter chain NPE or NPEC are more resistant to biodegradation than the longer chain NPE, but are ultimately biodegradable (Ahel et al 1996; APERC 1998; Staples et al 2001; Environment Canada 2001a). Di Corcia et al 1998 looked at the breakdown of the short chain carboxylate NP2EC and determined that the nonyl side chain can also becomes carboxylated forming various CNPEC forms. Castillo et al 2001, besides the formation of polyethylene glycol, NP2E and NP2EC as the major metabolites of NP9E photodegradation, also determined three minor products: a phenol ethoxycarboxylate (A_0PE_2C), a methylphenol ethoxycarboxylate (A_1PEC), and ethoxyphenyl heptanoic acid (CA_7PE)). Photodegradation of NP9E was slightly more efficient in wastewater, with a measured half-life of 14 hours, versus 17 hours in deionized water; NP9E disappeared over the 120 hours of the experiment (*ibid*). Manzano et al looked at the degradation of NP15E in river water and found that the metabolites were NP2E and ethylene glycol, then NP2EC and NP1EC; no NP1E was detected (Manzano et al 1998, 1999).

In a study by Staples et al, NPEC1, NPEC2, OPEC1, OPEC2, and NP were evaluated for biodegradability (Staples et al 1999). Half-lives for NPEC1 and NPEC2 in bacteria-seeded water were in the range of 18-22 days. The authors consider these to all be readily biodegradable based on CO_2 formation (a measure of ultimate mineralization). All compounds exceeded 60% of theoretical CO_2 within 28 days. The authors considered these compounds not to be persistent in the environment. In a follow-up study, Staples et al utilized two standardized European testing protocols to measure the biodegradation of NP9E, NP1.5E, and NP (Staples et al 2001). These three compounds were also readily mineralized in microbial-seeded water. Calculated half-lives were determined to be 7-14 days for NP9E, 19 days for NP1.5E, and 8 days for NP (*ibid*). Mineralization in water can be temperature dependent; for example NP15E mineralization percentages ranged from 30% at 7° C to 70-90% at 25° C in river water (Manzano et al 1999). The authors determined that the delay in cooler water was due to an increased lag time in acclimation of the necessary biological systems (*ibid*).

The basic aromatic ring at the center of the NPE molecule appears to break apart prior to the loss of the final ethoxylate or carboxylate groups, hence the formation of NP is not likely in aerobic conditions (APERC 1998, 1999a, 1999b; Jonkers et al 2001; Tanghe et al 1999; Staples et al 1999). Therefore, NP is not expected to be liberated in the aerobic biodegradation of NPE (Ahel et al 1994a; Maki et al 1996; Miles-Richardson et al 1999).

In anoxic, or anaerobic conditions, such as has been found in incomplete sewage treatment, some NP would be produced from the breakdown of NPE (Ahel et al 1994a, 1994b; Bennett, Metcalfe 2000; Giger et al 1984; US EPA 1996). This is evidently the primary source for environmental detections of NP in natural waters. Even after the breakdown of NPE in sewage treatment, the amount of NP is still no more than 4% of the total NPE input load (Ahel et al 1994a), indicating that formation of NP is not an extensive process in anaerobic conditions (in this same study 70% of the input load of NPE is not detectable in any form after sewage treatment). NP is adsorptive to soil organic carbon, and therefore would likely not move through the soil or stream sediments if formed under anoxic soil conditions (Environment Canada 2001a; US EPA 1996). Such anoxic conditions are very unlikely in the upper layers of forest soils.

In studies in Canada, after application of NP directly to a forested environment (NP was once used directly as a surfactant), at a rate of 0.47 L/ha (0.05 gallons/ac), NP was immediately detectable in stream water (maximum of 9.1 ppb) but reduced to trace levels in 6 hours (dilution was assumed). The NP persisted on spruce foliage for about 30 days, with a foliar half-life of about 3 hours. There were no

detections in soil, although this may have been a function of analysis methods (LOD 0.1 ppm). It was assumed to be on the ground, at a rate of 0.48 $\mu g/cm^2$, based on the application rate. NP was characterized as being "quite non-persistent in foliage, stream water, and sediment under field conditions" (Sundaram et al 1980). In a follow-up lab study simulating field conditions, 1 ppm of NP was added to stream and pond water, some with sediment as well. In flasks open to the air, NP half-life was measured at 2.5 days. When bottom sediments were added, about 50% of the NP was bound to these sediments, but this bound NP eventually was broken down, with a half-life of about 30 days (Sundaram, Szeto 1981). In a study of NP applied every second day for 20 days to outdoor microcosms which achieved mean maximum concentrations of 5, 23, 76, and 243 µg/L (Heinis et al 1999), the concentration of NP decreased over time after dosing was completed. In this study, NP had an estimated half-life in water of 0.74 days (range of 0.3 to 1.2 days) and 95% of the NP was gone from water in about 14 days (range of about 6 to 22 days). In the presence of oxygen, NP is rapidly broken down into other components, primarily carbon dioxide and water (APERC 1999a; Topp, Starratt 2000) through microbial action. NP is also sensitive to photochemical degradation in shallow water (Ahel et al 1994c). In Staples et al 1999, a half-life for NP was calculated as 20 days in river water; the authors considered NP not to be persistent in the environment.

In summary, very little NP would be expected to arise in the environment as a result of the application of NP9E, and what little NP might arise would be largely bound to soil or sediments and remain immobile while being biodegraded through microbial action (half-life of hours to 100 days (Maguire 1999; Sundaram et al 1980; Sundaram, Szeto 1981; Heinis et al, 1999; Staples et al 2001). The more likely compounds to be formed in a forested environment would be the short chain carboxylates. Based on this pattern of breakdown, the compounds of concern are the short-chain carboxylates (NP1EC, NP2EC), rather than NP9E, NP or the short-chain ethoxylates (NP1-3E). NP1EC and NP2EC would remain in an aqueous state until they too are ultimately broken down (Staples et al 1999; Environment Canada 2001a).

Only one mammalian toxicity study was found involving NP1EC, in which maternal oral exposure in rats via drinking water at 100 ppb while weaning resulted in no effects to male offspring (Sharpe et al 1995). Using a recombinant estrogen yeast screen assay, Routledge and Sumpter 1996 indicated that NP1EC and NP2EC showed a dose-dependent elevation in estrogenic effects, and were intermediate in estrogenic effects between NP and NP2E (approximately 25,000 times less potent than estradiol).

3.2.3.3.1. Acute Exposure

The three acute exposure scenarios assume that a young child (2- to 3-years old) consumes 1 L of contaminated water either after an accidental spill of 200 gallons of a field solution into a pond that has an average depth of 1 m and a surface area of 1000 m^2 or from a stream contaminated through drift, runoff or percolation or from overspray of a stream reach. Because these scenarios are based on the assumption that exposure occurs shortly after the spill, no dissipation or degradation of NP9E is considered.

The method used for determining exposure levels as a result of drift, percolation, or runoff can utilize data from operational water monitoring of herbicides in Region 5 of the Forest Service (Bakke 2001). NPE-based surfactants are commonly used with glyphosate formulations in Region 5. Although most water monitoring samples for glyphosate have been below the levels of detection, in some cases that limit has been relatively high (25 ppb). (The few positive detections have been below 25 ppb.) If the assumption is made that glyphosate could be occurring in surface water at the detection limit of 25 ppb

and that this would be the result of drift, runoff, or percolation, then the assumption could be made that the surfactant would also be in the water at an amount proportional to glyphosate. Commonly glyphosate is applied at 2% dilution (up to 4%), while the surfactant is applied at 1% (with a range of 0.25 to 2.5%). This would indicate that the surfactant could be present in water at a rate of 12.5 ppb (with a range of 3.1 to 31.2 ppb). These figures are comparable to those found in the monitoring studies discussed above, and will be used in the ambient stream scenarios.

Worksheet 1 in Appendix 1 describes an overspray scenario involving a stream reach. The levels in this worksheet will be used in the overspray scenario. As a way of reinforcing these values, in a recent study involving the aquatic application of R-11® surfactant (80% NP9E) and glyphosate to ponds for the control of aquatic weeds, NP9E and NP were immediately detectable after treatment at rates of 1,090 ppb and 15 ppb respectively (Trumbo, personal communication, 2002). After 8 days, the levels of NP9E and NP had dropped to 3.73 ppb and 0.57 ppb respectively (ibid).

The spill scenario is an extremely conservative scenario dominated by arbitrary variability. The actual concentrations in the water would depend heavily on the amount of compound spilled, the size of the water body into which it is spilled, the time at which water consumption occurs relative to the time of the spill, and the amount of contaminated water that is consumed. It also assumes instantaneous and complete mixing of the chemical into the waterbody.

3.2.3.3.2. Longer-Term Exposure

There has been no monitoring of NP9E or its metabolites in forested environments. There is considerable monitoring data that can define 'background' levels of contamination - i.e., levels in water that are not associated with specific applications of NP9E. Although these monitoring studies are generally associated with downstream waters, they can provide a conservative estimate of levels of NP9E and metabolites.

NP and NPE have both been detected in natural waterways in the US and Europe (Renner 1997). In one study, water samples were taken from a random sample of thirty rivers across the US; the highest level of NPE detected was 14.9 ppb, and NPE was found in 24% of the samples. In this same study, NP was detected in 30% of the samples; the highest detected amount of NP was less than 1 ppb (APERC 1999a; US EPA 1996). The highest levels were found in rivers considered heavily polluted or influenced by sewage treatment plants. In a study from the Lower Hudson River, NP detections ranged from 0.01 to 0.09 ppb (Dachs, et al 1999). In other studies, detections of NP and NPE have been normally associated with outfalls from sewage treatment plants or industrial facilities such as paper/pulp mills. Sewage treatment plants range in effectiveness in their ability to remove NP and NPEs from sewage (Ahel et al 1994a; 1994b; 1996; APERC 1999a; Bennett, Metcalfe 2000; Dentel et al 1993; Maguire 1999; Maki et al 1996).

As regards the potential for groundwater contamination, in Naylor et al 2000, NP9E was applied into two different individual household septic systems, one in a poorly-drained soil (leach field), one in a highly permeable soil (dry well). A bromide tracer was added as well. The bromide tracer took 5 months to reach 1.2 meter depth at the first site and 3 weeks to reach groundwater (at 10 m depth) at second site. Water collected from unsaturated zone lysimeters and from groundwater was monitored for NPE, NPEC, and NP. After 25 months at first site and 16 months at second site, no detections of NP, NPE, or NPEC were found in groundwater, indicating these do not migrate far from outlets in the subsurface, probably degrading in soil.

Sampling in sites removed from sewage treatment plants and mills are more representative of potential background levels in forests. Bennie, 1999, compiled data from sampling studies in Canada and the U.S. This monitoring showed that NP3-17E ranged from <1.6 to 14.9 µg/L. NP1EC ranged from non-detectable (ND) to 2 µg/L and NP2EC ranged from ND to 12 µg/L. NP levels ranged from ND to 2 µg/L in river water and from <0.002 to 72 µg/g (dry weight) in sediments (Bennie 1999). NP1E ranged from <0.02 to 7.8 µg/L in water and from <0.002 to 38 µg/g in sediments. NP2E ranged from <0.02 to 10 µg/L in water and <0.015 to 6.0 µg/g in sediments. The highest levels reported in Bennie 1999 for NP, NP1E and NP2E were from Hamilton Harbor, Ontario, downstream from the discharge of a sewage treatment plant.

For chronic exposure scenarios involving water, the cumulative levels of NP1-2EC as derived from Bennie 1999 will be used as the upper level (14 µg/L), with zero being the lower level and the midpoint of 7 µg/L being the central value.

3.2.3.4. Oral Exposure from Contaminated Fish

Some chemicals, particularly those that are poorly soluble in water and highly soluble in organic material, may be concentrated or partitioned from water into the tissues of animals or plants in the water. This process is referred to as bioconcentration. Generally, bioconcentration is measured as the ratio of the concentration in the organism to the concentration in the water. As with most absorption processes, bioconcentration depends initially on the duration of exposure but eventually reaches steady state. NP9E and its major environmental metabolites, NP1-2EC, however, are highly soluble in water and poorly soluble in organic material. Thus, for this risk assessment a bioconcentration factor of 1 L/kg is used. NP has been determined to bioconcentrate to some degree in fish (refer to section 4.1.3.1) but since NP9E and NPEC are the compounds of interest in aquatic environment, this is not critical to the assessment of risk.

For both the acute and longer-term exposure scenarios involving the consumption of contaminated fish, the water concentrations of NP9E and its metabolites are identical to the concentrations used in the contaminated water scenarios (see section 3.2.3.4). The acute exposure scenario is based on the assumption that an adult angler consumes fish taken from contaminated water shortly after an accidental spill of 200 gallons of a field solution into a pond that has an average depth of 1 m and a surface area of 1000 m^2 or about one-quarter acre. No dissipation or degradation is considered. Because of the available and well-documented information and substantial differences in the amount of fish caught and consumed by the general public and native American subsistence populations, separate exposure estimates are made for these two groups. The chronic exposure scenario is constructed in a similar way.

3.2.3.5. Oral Exposure from Contaminated Vegetation

None of the Forest Service applications of NP9E will involve the treatment of food crops. Thus, under normal circumstances and in most types of applications conducted as part of Forest Service programs, the consumption of vegetation contaminated with NP9E is unlikely. Nonetheless, any number of scenarios could be developed involving either accidental spraying of crops or the spraying of edible wild vegetation, like berries. In most instances, and particularly for longer-term scenarios, treated vegetation would probably show signs of damage from exposure to the herbicide used along with the NP9E surfactant, thereby reducing the likelihood of consumption that would lead to significant levels of human exposure.

Notwithstanding that assertion, it is conceivable that individuals could consume contaminated vegetation. One of the more plausible scenarios involves the consumption of contaminated berries after treatment of a right-of-way or some other area in which wild berries grow. The two accidental exposure scenarios developed for this exposure assessment include one scenario for acute exposure and one scenario for longer-term exposure. Both are based on similar analyses given in recent Forest Service national risk assessments (example SERA 1999). In both scenarios, the concentration of NP9E on contaminated vegetation is estimated using the empirical relationships between application rate and concentration in vegetation developed by Hoerger and Kenaga 1972 (as referenced by SERA 1999). For the acute exposure scenario, the estimated residue level is taken as the product of the application rate and the residue rate.

For the longer-term exposure scenario, a duration of 90 days is used - i.e., a fruit bearing plant is treated on day 0 and consumed by an individual over a 90-day post-treatment period. For this exposure scenario, the rate of decrease in the residues over time is taken from the vegetation half-time of 1 day (spruce foliage, utilizing NP, reported by Sundaram et al 1980).

For the acute exposure scenario, it is assumed that a woman consumes 1 lb (0.4536 kg) of contaminated fruit. Based on statistics from U.S. EPA this consumption rate is approximately the mid-range between the mean and upper 95% confidence interval for the total daily vegetable intake for a 64 kg woman. The range of exposures presented in Table 3-2 is based on the range of concentrations on vegetation from Hoerger and Kenaga 1972 and the range of application rates for NP9E. The longer-term exposure scenario is constructed in a similar way, except that the estimated exposures include the range of vegetable consumption from U.S. EPA as well as the range of concentrations on vegetation, and the range of application rates for NP9E.

Table 3-2: NPE - Summary of Exposure Scenarios for the General Public

Scenario	Target	Dose (mg/kg/day or event) Typical	Lower	Upper
Accidental/Incidental Exposures				
Directed spray, entire body	Child	0.002	0.00014	0.065
Direct spray, lower legs	Woman	0.0002	0.000015	0.0066
Dermal, contaminated vegetation	Woman	0.00031	0.0000036	0.018
Contaminated fruit, acute exposure	Woman	0.02	0.002	1.25
Contaminated water, spill	Child	0.46	0.14	1.71
Contaminated water, stream, drift, etc.	Child	0.00094	0.00014	0.0035
Contaminated water, stream overspray	Child	0.11	0.0069	0.55
Consumption of fish, general public	Man	0.014	0.0068	0.034
Consumption of fish, subsistence	Man	0.067	0.033	0.17
Chronic/Longer Term Exposures				
Contaminated fruit	Woman	0.00032	0.000032	0.020
Consumption of water	Man	0.0002	0	0.00048
Consumption of fish, general public	Man	0.000001	0.0	0.000002
Consumption of fish, subsistence	Man	0.0000081	0.0	0.000016

3.3 Dose-Response Assessment

3.3.1 Overview

At present there are no existing State or Federal human exposure guidelines for NP9E or NP. The use of the NOEL value of 10 mg/kg/day for NP from the study by Nagao et al 2001 would be a conservative value for protection against both NP and NPEs. Environment Canada 2001a used the Chapin et al 1999 LOEL value of 12 mg/kg/day as a functional NOAEL value. Utilizing a 10X safety factor for interspecies differences and a 10X safety factor for intraspecies differences provides a value of 0.10 mg/kg/day which should be protective of human health from chronic exposures to NP and NPEs. Short-term, or acute exposures to NP9E in the range of 0.1 to 0.4 mg/kg/day should not be associated with adverse health effects. The assessment level of 0.10 mg/kg/day should be protective of estrogenic or reproductive effects.

3.3.2 Existing Guidelines

At present there are no existing State or Federal human exposure guidelines for NP9E or NP. U.S. EPA has not established a Reference Dose (RfD)[8]. Since it appears that NP could be a component of the NP9E mixture (refer to section 2.2.1), NP could be an eventual degradation product of NP9E, and that NP appears to be more toxic in mammalian systems, one method of establishing a human threshold value would be to utilize NP toxicity studies to establish a benchmark level for use in assessing risks of exposure. As stated in Section 3.1.3, the use of the LOEL value of 12 mg/kg/day for NP from the study by Chapin et al 1999 as a functional NOAEL value is the approach utilized by Environment Canada 2001a. However, the more recent multigeneration study by Nagao et al, 2001, provides a NOEL value of 10 mg/kg/day for NP. Utilizing a 10X safety factor for interspecies differences and a 10X safety factor for intraspecies differences provides a value of 0.10 mg/kg/day which should be protective of human health from chronic exposures to NP and NPEs. Since the toxicity of NPEs decreases with increasing numbers of ethoxylate groups (refer to sections 3.1.2 and 3.1.3), and that the general population is exposed to mixtures that include NPEs of longer chain lengths, this protective value, based on NP, should be considered conservative. Another method would be to utilize the experimental values for NP9E, with the assumption that any testing involving the NP9E mixture would include minor amounts of NP and the short-chain NPEs. However there are few chronic tests available for consideration that involve NP9E. Of the two unpublished NP9E studies from Smyth and Calandra, 1969, only one provides dose values directly applicable to this risk assessment, with a NOEL of 28 mg/kg/day from a study in beagles. Hence the value of 0.10 mg/kg/day for NP will be used to assess risks of chronic human exposure.

As regards the estrogenicity or reproductive effects of NP and NPEs, based on studies described in section 3.1.4, it appears that most estrogenic effects are seen at relatively high dosages in mammals. For example, renal effects were observed at approximately 3 times lower doses of NP than effects in estrogen-responsive tissues in the multi-generation reproduction study in rats (Chapin et al 1999). In addition the estrogenicity of the NPEs is considerably lower than NP at the same doses. In the study by Nagao et al, 2001, a reproductive NOEL of 10 mg/kg/day was determined due to decreases in both relative and absolute ovary weights and an acceleration of vaginal opening in F1 and F2 females at the next highest dose of 50 mg/kg/day. It would appear that the assessment level of 0.10 mg/kg/day should be protective of any estrogenic or reproductive effects that NP and NPE exposures may represent in mammalian systems.

3.3.3 Dose-Response and Dose-Severity Relationships

As discussed in Section 3.2, several of the projected exposures for workers and the public exceed the derived RfD of 0.1 mg/kg/day. Table 3-4 summarizes the studies used to assess the dose-response and dose-severity relationships for NPE. Details for the studies summarized in this table are provided in Appendix 3, Table 1. In Table 3-4, all doses are expressed in units of mg chemical per kg body weight. A brief description of the effects noted at each dose level is included, and each effect is classified as a NOEL, Adverse Effect Level (AEL), or Frank Effect Level (FEL). For studies that involve exposing groups of male animals and female animals, comparable doses for the two groups are averaged and presented as a single entry if the effects in the two groups are qualitatively similar. As indicated in this

[8] The Reference Dose (RfD) is defined as a daily dose which is not anticipated to cause any adverse effects in a human population over a lifetime of exposure. These values are derived by the U.S. EPA.

table, several of the severity levels are associated with overlapping ranges of exposure. This is due to the uncertainties and variability in the available data.

For short-term exposures, 90-day subchronic tests involving NP9E in rats and beagles resulted in NOELs ranging from 10 to approximately 30 mg/kg/day. LOAELs from these same studies ranged upwards from 50 mg/kg/day. Slightly higher NOELs of 40 mg/kg/day were seen in 90-day subchronic studies with NP4E and NP6E. The use of the lowest sub-chronic NOEL of 10 mg/kg/day will be another conservative measure, considering that in these studies there is a considerable gap in dosing intervals between the NOEL and LOAEL levels determined in these studies. Again, using the same two safety factors as above, the human acute NOEL that will be used is 0.10 mg/kg/day. Based on the sub-chronic studies, however, short-term, or acute exposures to humans in the range of 0.1 to 0.4 mg/kg/day should not be associated with adverse health effects.

The available data on dogs, rats, and mice are relatively consistent, indicating that effects on the liver (weight increase, polysaccharide increase), kidneys (weight increase), or overall body weight decreases are the most sensitive endpoint for NP and NPE. At doses of 50 to 100 mg/kg/day, such changes would be expected.

For NPE, a dose of 250 mg/kg/day will be treated as a frank effect level in experimental mammals because of the decrease in litter size and increase in fetal rib defects seen in Meyer, 1988. For doses between 100 and 250 mg/kg/day, effects are uncertain in terms of seriousness, with inconsistent results in the various studies shown in Table 3-4. From the studies, it appears that dogs are more resistant to the effects of exposure than mice or rats.

Table 3-4. Summary of dose/response/severity data for NPE

Species (sex) Type of exposure, Compound	Dose (mg/kg/day)	Duration (days)	Effect Classification	Description of Effect	Reference
Rat, Dietary NP9E	10 50 250 1,250	90	NOEL AEL AEL AEL	No effects Decrease in liver polysaccharides In addition, Liver weight increase, kidney effects Same as above	Mellon Institute, 1960 Cited in Smyth and Calandra 1969
Rat, Dietary, NP9E	1 4 18 68 250 485	90	NOEL NOEL NOEL AEL AEL AEL	No effect No effects No effects. Weight loss Weight loss, emaciation, low body fat, poor organ development Same as above	Shelanski, 1960 Cited in Smyth and Calandra 1969
Rat, Dietary, NP9E	20 70 230	90	NOEL AEL AEL	No Effects Liver weight increase Liver, kidney, spleen weight increase; growth rate decrease	Dow Chemical, 1960 Cited in Smyth and Calandra 1969
Rats, Dietary NP6E	40 200 1,000	90	NOEL AEL AEL	No Effects Liver weight increase; male kidney weight increase Same as above, plus weight loss	Industrial Bio-Test Labs, 1963a
Dogs, Dietary, NP9E	12 170 1,200	90	NOEL AEL AEL	No effects Weight gain decrease Weight gain decrease	Shelanski, 1960
Dogs, Gavage, NP4E	40 200 1,000	90	NOEL AEL AEL	No effects Increase in liver weight; emesis Increase in liver weight; emesis; decrease in body weight	Industrial Bio-Test Labs, 1963c As cited in Johnson 1999
Dogs, Gavage, NP6E	40 200 1,000	90	NOEL AEL AEL	No effects Emesis Emesis; increase in female relative liver weight	Industrial Bio-test Labs, 1963b

Rats, Dietary, NP4E	40 200 1,000	730	NOEL AEL AEL	No Effects Female growth loss Growth loss; increase in relative liver weight	Smyth and Calandra 1969
Dogs, Dietary, NP9E	8.5 28 88	730	NOEL NOEL AEL	No effects No effects Increase in relative liver weight	Smyth and Calandra 1969
Rats, female Gavage, NP9E	50 250 500	10	NOEL FEL FEL	No effects Maternal weight decrease; decrease in litter size; fetal defects Same as above	Meyer, 1988
Mice, Female, Gavage, NP6E	600	8	NOEL	No effects	Hardin, 1987
Rats, Dietary, NP	15 50 150	90	NOEL NOEL AEL	No effects No effects Body weight decrease; kidney weight increase; male hyaline decrease	Cunny, 1997
Rats, Dietary, NP	22 65 225	Three generations	AEL AEL AEL	Relative kidney weight increase; kidney histopathology Same as above; acceleration of vaginal opening; decrease in body weight Same as above	Chapin, 1999
Rats, Gavage, NP	2 10 50	Two generations	NOEL NOEL AEL	No effects No effects Relative female kidney and liver weight increase; decrease in ovary weight; body weight decrease; liver and kidney histopathology; acceleration of vaginal openings; decrease in pups/litter	Nagao, 2001
Rats, Dietary NP	0.5 2 13, 22 35, 58 61, 110 127, 244	One generation	NOEL NOEL NOEL NOEL FEL FEL	No effects No effects No effects No effects Polycystic kidney disease in F1; decrease in maternal weight In addition to above, weight loss in F1	Latendresse, 2001
Rats, Gavage, NP	100 250 400	One generation	AEL AEL FEL	F1 male weight decrease; testicular mass decrease; effects to male testicular structure. In addition to above; decrease in body weight; decrease in sperm count No offspring born	DeJager et al 1999
Rats, Dietary, NP	3 65 220	One generation	NOEL AEL AEL	No effects F1 female body weight decrease F1 body weight decrease; increased sodium solution intake	Ferguson et al 2000

Based on the analysis above, and using the 100X safety factor to extrapolate to humans; exposures below the RfD should not represent any risk of effects. As noted above, the RfD is considered to be a daily dose at which no adverse effects are anticipated in a population over a lifetime exposure. The RfD is intended to be a conservative estimate and does not explicitly incorporate information on dose-duration or dose-severity relationships. In other words, doses below the RfD, regardless of the duration of exposure, are of no substantial concern as long as the RfD is based on a sound set of data. The assumption that exposures above the RfD will result in adverse human health effects is not necessarily correct, particularly when the duration of exposure is substantially less than lifetime.

All exposure scenarios considered in this risk assessment are less than a lifetime. As discussed above, short-term NPE exposures between 0.1 and 0.4 mg/kg/day (HQ values between 1 and 4) should also represent little or no risk to humans. NPE exposures between 0.5 and 1.0 mg/kg/day (HQ values between 5 and 10) may result in risks of kidney and liver effects, and could represent risks to developing fetuses in pregnant women. The risks to pregnant women at this level are based on NP exposure rather than NP9E, so can be considered a conservative interpretation of the data. NPE exposures between 1.0

and 2.5 mg/kg/day (HQ values between 10 and 25) will result in increasing risks of kidney, liver and growth effects, and increasing risks to the developing fetus in pregnant women. Exposures greater than 2.5 mg/kg/day (HQ values greater than 25) would represent a risk of frank toxic effects.

3.4 Risk Characterization

3.4.1. Overview

Based on the estimated levels of exposure and the criteria for acute and chronic exposures, there is no evidence that typical exposures to NP9E-based surfactants will lead to dose levels that exceed the level of concern. For workers, only the upper level of general exposure results in estimates of absorbed doses that exceed the derived RfD. It is unlikely that any worker would be utilizing such high levels of NP9E-based surfactants on a chronic basis; the high rate in this scenario is uncommonly used in the Forest Service. However, this does point out the need for good industrial hygiene practices when utilizing high levels of NP9E-based surfactant.

For members of the general public, the upper limits for hazard quotients for chronic exposures are below a level of concern and the risk characterization is relatively unambiguous. Based on the available information and under the foreseeable conditions of application, there is no route of exposure or scenario suggesting that the general public will be at any substantial risk from longer-term exposure to NP9E-based surfactants.

For the public, acute or accidental exposure scenarios involving consumption of contaminated water, consumption of contaminated vegetation, or subsistence consumption of fish represent some risk of effects. None of the other acute exposure scenarios represent a risk of effects to the public from NP9E exposure. At typical rates of application, the drinking of contaminated water after a spill could present a risk of subclinical effects to the liver and kidney. The exposure scenario for the consumption of contaminated water is an arbitrary scenario: scenarios that are more or less severe, all of which may be equally probable or improbable, easily could be constructed. The consumption of contaminated vegetation also represents a risk of clinical effects at the high application rates only. At the typical rate of application, the risk is considered acceptable. Nonetheless, this and other acute scenarios help to identify the types of scenarios that are of greatest concern and may warrant the greatest steps to mitigate. For NP9E, such scenarios involve oral rather than dermal exposure.

Irritation and damage to the eyes can result from exposure to relatively high levels of NP9E -i. e., placement of NP9E directly onto the eye - and repeated exposures to undiluted NP9E-based surfactants can lead to skin sensitization. From a practical perspective, eye irritation and skin sensitization are likely to be the only overt effects as a consequence of mishandling NP9E. These effects can be minimized or avoided by prudent industrial hygiene practices during the handling and application of NP9E-based surfactants.

There is the potential for exposures to other man-made and natural estrogen-like compounds. A consideration of potential cumulative effects is limited because of a lack of comprehensive information on the skin absorption kinetics of NPE in mammalian systems. Contributions to background exposures to other xenoestrogens from exposure to NPE may be negligible depending upon the background exposures, lifestyles, absorption rates, and other potential natural or man-made chemical exposures that are used to determine overall risk to environmental xenoestrogens.

U.S. EPA is completing testing protocols and setting priorities for testing for endocrine effects, as a result of the passage of the Food Quality Protection Act of 1996. As the body of research on endocrine effects continues to grow through this effort and others, the Forest Service will review new studies to determine whether any of the conclusions in this paper should change.

3.4.2. Workers

A quantitative summary of the risk characterization for workers associated with exposure to NP9E is presented in Table 3-5. The quantitative risk characterization is expressed as the hazard quotient, which is the ratio of the estimated doses from Table 3-1 to the derived RfD of 0.10 mg/kg/day (section 3.3.2).

Given the low hazard quotients for accidental exposure, the risk characterization is reasonably unambiguous. None of the accidental exposure scenarios exceed a level of concern. While the accidental exposure scenarios are not the most severe one might imagine (e.g., complete immersion of the worker or contamination of the entire body surface for a prolonged period of time) they are representative of reasonable accidental exposures. As discussed in section 3.2.2.2, however, confidence in this assessment is diminished by the lack of comprehensive information regarding the dermal absorption kinetics of NP9E in humans. Nonetheless, the statistical uncertainties in the estimated dermal absorption rates, both zero-order and first-order, are incorporated into the exposure assessment and risk characterization.

The upper limit of general exposure scenarios for all methods of application results in a modest excursion above the derived RfD for backpack and aerial application (HQ = 5) while the HQ for boom spray operations results in an HQ of 10. As discussed in section 3.2.2.1, these upper limits of exposure are constructed using the highest anticipated application rate, the highest anticipated number of acres treated per day, and the upper limit of the occupational exposure rate. If any of these conservative assumptions were modified (e.g., the compound is applied at the typical rather than the maximum application rate) the hazard indices would be at or below unity. Given the conservative nature of the RfD itself, it is unlikely that there would be any signs of toxicity. In addition, as discussed in section 3.3.2, non-chronic exposures to NP9E up to 0.4 mg/kg/day should not result in toxic effects. As discussed in Section 3.3.3, there is a risk of kidney and liver effects from exposures at these levels. The simple verbal interpretation of this quantitative characterization of risk is that under the most conservative set of exposure assumptions, workers could be exposed to levels of NP9E that are regarded as unacceptable. If NP9E is not applied at the highest application rate or if appropriate steps are taken to ensure that workers are not exposed at the maximum plausible rates (i.e., worker hygiene practices and/or reduced areas of treatment per day) there is no indication that the workers would be at risk of incurring systemic toxic effects.

As discussed in section 3.1.6, NP9E can cause irritation and damage to the skin and eyes. Quantitative risk assessments for irritation are not derived; however, from a practical perspective, eye or skin irritation is likely to be the only overt effect as a consequence of mishandling NP9E. These effects can be minimized or avoided by prudent industrial hygiene practices during the handling of NP9E.

Table 3-5: NPE - Summary of Risk Characterization for Workers

Acute RfD	0.1	mg/kg/day	Section 3.3.2
Chronic RfD	0.1	mg/kg/day	Section 3.3.2
	Hazard Quotient		
Scenario	Typical	Lower	Upper
General Exposures			
Directed ground spray (backpack)	0.1	0.0008	5
Boom Spray	0.4	0.001	10
Aerial Applicaion	0.01	0.0004	5
Accidental/Incidental Exposures			
Immersion of Hands, 1 minute	0.002	0.0003	0.01
Contaminated Gloves, 1 hour	0.1	0.02	0.7
Spill on hands, 1 hour	0.0005	0.00004	0.02
Spill on lower legs, 1 hour	0.001	0.0001	0.04

3.4.3. General Public

The quantitative hazard characterization for the general public is summarized in Table 3-6. Like the quantitative risk characterization for workers, the quantitative risk characterization for the general public is expressed as the hazard quotient using the derived RfD of 0.10 mg/kg/day.

Although there are several uncertainties in the longer-term exposure assessments for the general public, as discussed in section 3.2, the upper limits for hazard indices are sufficiently far below a level of concern that the risk characterization is relatively unambiguous: based on the available information and under the foreseeable conditions of application, there is no route of exposure or scenario suggesting that the general public will be at any substantial risk from longer-term exposure to NP9E.

For the acute/accidental scenarios, exposure resulting from the consumption of contaminated water is of greatest concern, exposure resulting from the consumption of contaminated vegetation is of somewhat less concern, and subsistence consumption of fish is of marginal concern. None of the other acute exposure scenarios represent a risk of effects to the public from NP9E exposure.

The spill scenario represents the greatest risk, with an HQ of 5 for the typical application rate, and an HQ exceeding unity even with the lowest application rates. As discussed above, an HQ of 5 represents a risk of subclinical effects to the liver and kidney. The upper HQ of 17 represents an increasing risk of clinical effects to the kidney, liver, and other organ systems. As discussed in some detail in section 3.2.3.4, the exposure scenario for the consumption of contaminated water is an arbitrary scenario: scenarios that are more or less severe, all of which may be equally probable or improbable, easily could be constructed. All of the specific assumptions used to develop this scenario have a simple linear relationship to the resulting hazard quotient. Thus, if the accidental spill were to involve 20 rather than 200 gallons of a field solution of NP9E, all of the hazard quotients would be a factor of 10 less.

The consumption of contaminated vegetation also represents a risk of clinical effects at the high application rates only (HQ = 12). At the typical rate of application, the HQ is less than one. Nonetheless, this and other acute scenarios help to identify the types of scenarios that are of greatest concern and may warrant the greatest steps to mitigate. For NP9E, such scenarios involve oral rather than dermal exposure.

Table 3-6: NPE - Summary of Risk Characterization for the General Public

Acute RfD	0.1	mg/kg/day	Section 3.3.2	
Chronic RfD	0.1	mg/kg/day	Section 3.3.2	
		Hazard Quotient		
Scenario	Target	Typical	Lower	Upper
Accidental/Incidental Exposures				
Directed spray, entire body	Child	0.02	0.002	0.6
Direct spray, lower legs	Woman	0.002	0.0002	0.07
Dermal, contaminated vegetation	Woman	0.003	0.00004	0.2
Contaminated fruit, acute exposure	Woman	0.2	0.02	12
Contaminated water, spill	Child	5	1.4	17
Contaminated water, drift, etc.	Child	0.009	0.001	0.04
Contaminated water, overspray	Child	1.1	0.07	6
Consumption of fish, general public	Man	0.1	0.07	0.3
Consumption of fish, subsistence	Man	0.7	0.3	2
Chronic/Longer Term Exposures				
Contaminated fruit	Woman	0.003	0.0003	0.2
Consumption of water	Man	0.002	0	0.005
Consumption of fish, general public	Man	0.00001	0	0.00002
Consumption of fish, subsistence	Man	0.00008	0	0.0002

3.4.4. Sensitive Subgroups

There is limited information to suggest that specific groups or individuals may be especially sensitive to the systemic effects of NP9E-based surfactants. As indicated in section 3.1.3, NP9E can cause decreased body weight, increases in kidney and liver weight, and effects to kidney function and structure. Thus, individuals with pre-existing conditions that involve impairments of the kidney or liver may be more sensitive to this compound. There is some indication that sensitive individuals may develop contact allergies based on scattered case histories (refer to section 3.1.6). People with a history of skin allergic reactions to soaps and detergents may be especially sensitive to dermal exposures of NP9E-based surfactants.

The potential of NP9E to induce reproductive effects described in section 3.1.4, should be considered low. Based on the available dose/duration/severity data, it appears that exposure levels below those associated with the most sensitive effect (i.e., kidney effects) are not likely to be associated with reproductive toxicity. However, as shown in the exposure scenarios, there is the potential for acute exposures to be in the range (considering a 100X safety factor) where effects to the developing fetus may occur, therefore pregnant women could be considered a sensitive population.

3.4.5. Connected Actions

NP9E-based surfactants are intended to be mixed with herbicides and applied as a tank mix. In the literature review conducted in USFS 2002, there appears to be little in the scientific literature suggesting that NP9E-based surfactants will interact, either synergistically or antagonistically, with the herbicides commonly used in forestry in Region 5, in regards to human health effects. These types of surfactants don't appear to increase dermal absorption of the herbicides (*ibid*) (also refer to Nielsen and Andersen 2001). Synergistic effects are not expected from multiple exposures to NP, NPEs, and their breakdown products (Payne et al 2000, Environment Canada 2001a).

3.4.6. Cumulative Effects

As noted above, this risk assessment specifically considers the effect of repeated exposure in that the chronic (derived) RfD is used as an index of acceptable exposure. As discussed in the dose-response and dose-severity relationships (see section 3.3.2), the daily dose rather than the duration of exposure appears to determine the toxicological response. Consequently, repeated exposure to levels below the toxic threshold should not be associated with cumulative effects. However, estrogenic effects can be caused by additive amounts of NP, NPE, and their breakdown products. In other words, an effect could arise from the additive dose of a number of different xenoestrogens, none of which individually have high enough concentrations to cause effects (Bolt 2001; Environment Canada 2001a; Payne et al 2000; US EPA 1996). This can also extend out to other xenoestrogens that biologically react the same. Additive effects, rather than synergistic effects, are expected from combinations of these various estrogenic substances (Payne et al, 2000; Thorpe et al, 2001).

When assessing cumulative effects of exposure to NP and NPEs, there must be some consideration of the contribution from other sources, such as personal care products (skin moisturizers, makeup, deodorants, perfumes, spermicides), detergents and soaps, foods, and from the environment away from the forest herbicide application site. In Environment Canada 2001a, the authors made some estimates of these background exposures based on extrapolation of admittedly limited data and very conservative assumptions. One of the more critical, and extreme, assumptions made was that dermal absorption of NP and NPEs would be 100%. This assumption was based on the inadequacy of the one *in vitro* study of absorption in human skin (refer to section 3.1.7). However, this would seem to be an extreme over-assessment of absorption and leads to estimates of exposure that greatly exceed the threshold value for kidney damage in rats from NP exposure of 12 mg/kg/day. In APERC 2000, the skin absorption of NP, NP4E, and NP9E is discussed and an unpublished study is described that supports the results from the previous study on *in vitro* human skin absorption described in section 3.1.7. Based on a review of the literature on surfactants and absorption, USFS 2002, it would appear that a 100% figure is extremely conservative. The use of a 1% absorption rate would appear to be a realistic figure; the 100% figure will be considered a worst-case figure.

Contributions from the air, water, soil, and food of NP and NPEs in adult Canadians was estimated at 0.034 mg/kg/day (Environment Canada 2001a). The contribution of NP and NPEs from the exposure to skin moisturizers, makeup, deodorant, fragrances, detergents, cleaners, paints, and spermicides are also estimated in Environment Canada 2001a. Both of these exposure sources are based on very small sample sizes and should be considered worst-case. Using the skin absorption figure of 100%, and the highest concentration estimates, these products contribute up to 27.0 mg/kg/day, assuming each is used every day. If a 1% dermal absorption figure is used, this total would be reduced to 0.27 mg/kg/day. In a study from Europe, the daily human exposure to NP is estimated at 0.002 mg/kg/day (2 μg/kg/day) as a worst-case assumption (note that this estimate does not include the ethoxylates) (Bolt 2001). In the study by Muller and Schlatter (1998) the estimated daily oral intake of NP from food, water, and plastic food packaging was estimated at 0.16 mg/day (note that this is not on a body weight basis; converting to a per kg basis would result in similar values to Bolt 2001).

In addition to xenoestrogens, humans are exposed to various phytoestrogens, which are hormone-mimicking substances naturally present in plants. Specific compounds that have been identified as phytoestrogens include coumestrol, formononetin, daidzein, biochanin A, and genistein (US EPA 1997). In all, more than 300 species of plants in more than 16 families are known to contain estrogenic substances, including beets, soybeans, rye grass, wheat, alfalfa, clover, apples, and cherries (*ibid*). Background exposures of Europeans to natural phytoestrogens (isoflavones (daidzein, genistein) and lignans), mainly from soybeans and flaxseed, is estimated at 4.5-8 mg/kg body weight for infants on soy-based formulae (Setchell et al 1997), and up to 1 mg/kg body weight for adults (Cassidy 1996). In East Asian populations where soy-based foods are more commonly consumed, estimates of intake of phytoestrogens are in the range of 50-100 mg/kg/day (Cassidy, 1996; Degen 1999 as referenced in Bolt 2001). Some might consider that the contribution from these natural phytoestrogens should be disregarded, as the human species has adapted over time to daily exposures to such compounds. However, at a biochemical level, these phytoestrogens can react similarly to the estrogenic xenoestrogens, such as NP. There is some indication that a soy-based diet could act to ameliorate the effects of exposure to NP (Latendresse, et al, 2001).

From section 3.1.4, based on the studies by Chapin et al and Nagao et al, the lowest reproductive NOEL for NP is 10 mg/kg/day from these studies in rats. Assuming a 100X safety factor to convert to a human reproductive NOEL would result in a value of 0.10 mg/kg/day. Adding the contributions from the worst-case background environment and consumer products, as described in Environment Canada 2001a, there would be a background dose to a female worker, of 27.034 mg/kg/day (assuming 100% dermal absorption) or 0.304 mg/kg/day (assuming 1% dermal absorption. Using the derived NP human NOEL of 0.10, these exposure estimates result in hazard quotients of 270 to 3. In terms of this risk assessment, the contribution of NP9E (workers exposure ranged from 0.000075 to 1.01 mg/kg/day) would contribute from 0.00075 up to 10 to any hazard quotient. This may be negligible depending upon the background exposures, lifestyles, absorption rates, and other potential natural or man-made chemical exposures that are used to determine overall risk to environmental xenoestrogens.

4. Ecological Risk Assessment

4.1 Hazard Assessment

4.1.1 Overview

The toxicity data set for NP is relatively large. There are many studies reporting acute and chronic effects of NP, fewer studies reporting the toxicity of NP9E, and only a few studies that included the NPECs. Although studies in the literature have used many species, different test methods, and different chemicals, there is a consistent pattern in the toxicity. NP is more toxic to fish, invertebrates and algae than NPEs. There is an increase in toxicity of NPEs with decreasing number of ethoxylate groups. NPECs are less toxic than the corresponding NPE (NP1EC vs NP1E) and have toxicities similar to those of NPEs with 6 to 9 ethoxylate units. The relative toxicities differ from the relative estrongenicities. NPEC is much less acutely toxic relative to NP but has only slightly lower estrogenic potency. Since the relative potencies of the various compounds is based on *in vitro* trout hepatocyte studies, not whole organism studies, caution in interpretation is necessary.

4.1.2 Toxicity to Terrestrial Organisms

4.1.2.1. – Mammals

Refer to the discussion on toxicity to humans as most of the experimental data is based on mamallian toxicity tests. Refer also to Table 3-4 as well as Table 1 in Appendix 3.

4.1.2.2 – Birds

There is no data in the published literature on the effects of NP or NPEs to birds. In APERC 2000, there is reference to an unpublished study on bobwhite quail. This study exposed quail chicks to NP9E in the diet for 5 days at concentrations from 0 to 5,000 ppm with 3 days of additional observation. There were no effects seen in behavior, nor was there any mortality. The authors concluded that the LC_{50} was greater than 5,000 ppm.

4.1.2.3. – Terrestrial Invertebrates

Tests on the earthworm (*Apporectodea calignosa*) indicated a 21-day EC10 (reproduction) of 3.4 µg/g in soil for NP (Krogh et al., 1996 as cited in Environment Canada 2001a).

With the exception of one study, there have been no tests of NP or NPE on terrestrial insects that were found in the literature, beyond that found for the aquatic nymph stages as discussed in Section 4.1.3.3.

One study involving terrestrial invertebrates involves a study of honeybees conducted in New Zealand. This study (Goodwin and McBrydie, 1999) involved the application of unrealistically high spray volumes to anesthetized bees placed in a petri dish. The study tested several surfactants, including two that are alkylphenol polyethoxylate-based (Citowett and Multifilm) however the exact formulations are not specified. In the case of Citowett, it was applied at concentrations up to 0.1% in water at a rate equivalent to 250 gallons per acre, while Multifilm was applied up to 0.1% in water at 205 gallons per acre. Although Citowett, at concentrations above 0.031% resulted in increased levels of mortality over controls, due to such high volumes of water and surfactant applied to anesthetized bees, there is a strong chance that any lethal effect was due to drowning, rather than the toxicity of the surfactant. Because of

the study design, Goodwin and McBrydie does not provide sufficient data to characterize the risk to terrestrial invertebrates.

4.1.2.4. – Terrestrial Plants (Macrophytes)

There is only limited data on the toxicity of NP to plants, and there is no data in the published literature on NPEs. Research by Bokern and Harms indicated that the concentration of NP causing a 50% reduction in cell suspension cultures of 14 species ranged from 0.05 mM (11 mg/L) to more than 1.00 mM (220 mg/L) (Bokern and Harms, 1997). Of two lupine species, root cultures were affected by NP; one species did not reach 50% growth reduction at NP levels in soil up to 220 mg/L while the other showed a 50% reduction at 22 ppm (Bokern et al 1998). The uptake of NP from the soil was slow, and NP was quickly mineralized by soil microorganisms (*ibid*). NP was taken up by plant roots from the soil, however no more than 1% was found in the roots or shoots after 21 days, and was metabolized to hydroxylated and conjugated derivatives (*ibid*). In a recent study from Denmark (Mortenson and Kure, 2003), soils were contaminated with NP by incorporating domestic sewage sludge, or directly by spiking with NP at rates from 13 to 534 ppb, dry weight. Rape plants (*Brassica napus*) were then grown in this contaminated medium. No uptake of NP was seen (LOD = 100 ppb, dry weight) in the plants, nor did the NP have any effect on plant biomass.

Since NP9E-based surfactants would not be applied alone, but would be applied in a mix with an herbicide, the herbicide would determine the effects to terrestrial plants. As such, there will be no further discussion of NP9E effects to terrestrial plants.

4.1.2.5.- Terrestrial Microorganisms

Existing soil microbes are able to utilize NPE and NP with little or no lag phase (Environment Canada 2001a; Topp 2000), at application rates (of NP) in the soil of from 1 to 250 mg/kg, indicating a lack of toxicity to soil microorganisms.

4.1.3. Toxicity to Aquatic Organisms

There has been concern expressed about the aquatic toxicity of NPE and NP. Similar to the results on mammals, studies have shown that the direct toxicity of NP may be of more concern than any estrogen-like effects (Schwaiger et al 2000). The acute and chronic toxicity of NP9E is one to three orders of magnitude less than NP, while the toxicity of the intermediate breakdown products, NPEC and shorter chain NPEs, are intermediate between NP and NPE (Baldwin et al 1998; Environment Canada 2001a; US EPA 1996). In general, the relative toxicity can be shown as NP > NP1-3E > NP1-3EC > NP4-9E (Environment Canada 2001a). Although acute and chronic toxicity increases as ethoxylate groups are removed during NP9E breakdown, overall there is less NP-based compound amounts due to ultimate degradation of the NP molecule (Servos 1999; Environment Canada 2001a). In a die-away study (Yoshimura, 1986), there was an observed net reduction in toxicity despite the degradation of NPEs to more toxic, lower molecular-weight constituents.

A stated in section 3.2.3.3, the compounds of environmental interest in aquatic environments are more likely to be the short chain carboxylates of NPE (NP1EC and NP2EC). One study showed that NP1EC was slightly more estrogenic than NP in two of three *in vitro* tests (White 1994), however, in general, the relative estrogenic potency can be shown as NP > NP1-2E > NP1-2EC > NP9E (Environment Canada 2001a). NP1-2E and NP1-2EC are expected to be only slightly less estrogenic then NP (Jobling, Sumpter 1993). Because of the potential importance of the NP1-2EC compounds in the forested

environment, the estrogenic response of these compounds is of concern. As shown above, there is some discrepancy on the relative estrogenicity of these compounds. Different tests have shown widely different results; and although the different results may be due to the different assays, it does raise some uncertainty concerning the relative estrogenicity of these compounds.

Vitellogenin

Vitellogenin is a protein occurring naturally in the serum of adult female nonmammalian vertebrates during egg production. Vitellogenin is the precursor molecule for egg yolk. The liver synthesizes and secretes vitellogenin, which is then carried in the bloodstream to the oocytes, or egg cells. The developing oocytes take up vitellogenin and convert it into egg yolk proteins. Estrogen, acting through the estrogen receptor gene, is the primary stimulus for vitellogenin synthesis and secretion. Vitellogenin production is normally restricted to adult females. However, vitellogenin production can be induced in males and immature females by exogenous administration of estrogen or estrogenic agents. Consequently, the presence of vitellogenin in the blood of male oviparous animals can serve as an indicator of exposure to xenobiotic estrogens (Palmer et al 1998; Billinghurst et al 2000). Some of the early concerns about NP and NPE were the result of elevated vitellogenin levels seen in male fish in British rivers (Renner 1997; Jobling 1998). In a study by White et al 1994, the authors looked at the comparative ability of OP, NP, NP2E and NP1EC to induce vitellogenin in rainbow trout hepatocytes. This *in vitro* study indicated that OP was much more potent than NP, with NP1EC and NP2E less potent than NP and roughly equivalent to each other. All four compounds indicated an estrogenic response. In a three-week flow through experiment with adult rainbow trout, NP, NP1EC, and NP2E all induced vitellogenin at levels of 30-40 µg/L (only single exposure levels were used) (Jobling et al 1996). In a second three-week flow through experiment, with the same age fish, NP induced vitellogenin at 20.3 µg/L, with a NOEC value of 5.02 µg/L (*ibid*). In a 72-hour flow through test using NP, with male and female rainbow trout, a 72-hour EC50 for vitellogenin induction was 14.14 µg/L (Lech et al 1996). In a 21-day flow-thru NP exposure to female juvenile rainbow trout, elevated vitellogenin was seen at 16 µg/L, with a NOEC of 6.7 µg/L (Thorpe et al 2000). In the same experiment, the authors found an increase in relative liver weight after 21 days of exposure to 19 µg/L or more NP. This is consistent with vitellogenin synthesis, which would result in an increased liver weight (*ibid*). In a 42-day intermittent flow-through dosing system, doses of 0.64 µg/L caused a detectable amount of vitellogenin to appear in serum of adult sheepshead minnows (Hemmer et al 2001). In a year-long exposure to NP at 1 and 10 µg/L, some, but not all of the exposed rainbow trout exhibited increased vitellogenin at levels as low as 1 µg/L (ppb) (Ackermann et al, 2002).

Elevated vitellogenin levels in males caused by exposure to NP will respond to decreases of NP exposure with a corresponding reduction in vitellogenin, however this response has a lag time. In a study by Hemmer et al 2002, male sheepshead minnows were exposed to NP at 6 or 60 ppb for 16 days, followed by monitoring in clean water for 96 days. The elevation of vitellogenin in the plasma peaked within 2-4 days of the cessation of exposure, and then slowly decreased, with a serum half-life of 13.3 days after exposure to the 60 ppb dose (*ibid*). The half-life of hepatic vitellogenin RNA was much shorter (1-2 days) with a 94-99% decrease within 8 days of exposure cessation (*ibid*).

There is often a poor correlation between elevated male vitellogenin levels caused by exposure to NP and male reproductive function. In a recently presented abstract and poster involving cunner (*Tautogolabrus adspersus*), a species of temperate reef fish, preliminary data indicate that viable sperm are still produced with blood vitellogenin levels approaching 400 mg/ml (Mills et al 2001). This

abstract found a correlation (although low, $R^2 = 0.17$) between female egg production and male plasma vitellogenin (*ibid*). In another study on Japanese medaka (*Oryzias latipes*), exposure to NP caused elevated vitellogenin in males, but there were no effects to fertilization ratios (Kashiwada et al 2001). In a study by Ackermann et al, 2002, exposures to NP at levels of 1 and 10 ppb over a year had no effect on rainbow trout hatching rate, body weight, sex ratios of offspring, or the development of testis/ova in males. The induction of vitellogenin may be the most sensitive endpoint for exposure to xenoestrogens, but it doesn't necessarily equate to reproductive harm to male fish at low levels. In a study in which estradiol was injected in male summer flounder, elevated vitellogenin levels were deemed responsible for histopathologic effects to the liver (hepatocyte hypertrophy), kidneys (obstruction or rupture of renal glomeruli) and testes (disruption of spermatogenesis) (Folmar et al 2001). An accumulation of a hyaline material in these structures was partially identified as vitellogenin (*ibid*).

In a recently presented abstract and poster, juvenile male trout were sampled from eight mid-Sierra Nevada lakes. These sampled male trout were examined for vitellogenin expression; no vitellogenin induction was found from trout at any site (below detectable limits) (McClain et al 2001). This could indicate low levels, or an absence, of xenoestrogens in these relatively pristine Sierran lakes.

Based on monitored amounts of NP in river water (0.6 µg/L in US, 45 µg/L in Switzerland) and the minimum thresholds for inducing vitellogenin in fish (10-50 µg/L – refer to Appendix 3, Table 2), it is likely that the induction of vitellogenin is not commonly occurring as a result of NP exposure in US waters, but could be an issue in Europe where NP levels can be higher as a result of less efficient sewage treatment. Based on these results, and the acute and chronic toxicity of NP, non-reproductive toxicity is the more critical effect. For example, the 90-day NOEC for NP in rainbow trout based on growth effects is 6 µg/L (Brooke, 1993, unpublished report, as cited in Servos, 1998; Staples et al 1998), while the minimum concentration for vitellogenin induction in the same species is 20 µg/L (Jobling et al 1996; Staples et al 1998).

In a recently presented abstract by Hecht et al 2001, the authors compared the exposure to NP of juvenile salmon being directly exposed in water or indirectly exposed after feeding on amphipods after the amphipods were fed NP. The indirect dose of NP from amphipods was 1.5 to 7.3 µg/g wet weight per fish. The direct NP dose in water ranged from 0 to 500 µg/L for 96 hours. There were no increases in vitellogenin after feeding on exposed amphipods, but significant increases after being exposed to water at 60 µg/L. Waterborne exposures to NP appear more potent in stimulating vitellogenin than indirect dietary exposures to NP.

Vitellogenin is also present in frogs and toads. In an abstract of a study by Selcer et al 2001, vitellogenin increases were not seen in adult male leopard frogs after exposure to NP or OP at 1 mg/L for 20 days. NP also did not induce vitellogenin in *Xenopus laevis* after exposures of 1 ppm NP (1 mg/L) for 14 days. The author believes that fish species are more sensitive to NP and OP than frogs (*ibid*).

Vitellogennic animals could be particularly sensitive to metal exposure as vitellogenin can bind to metals (Crain, Guillette 1997). As regards 14-day survival however, a study involving NP and copper exposure to rainbow trout showed an antagonistic effect; another study of NP and cadmium exposure to rainbow trout indicated a lack of synergism in metal transfer in the gills (both studies cited in Lewis 1992, Table 5; Calamari and Marchetti, 1973).

4.1.3.1. – Fish (Refer also to Table 2 in Appendix 3)

In terms of NP9E acute toxicity, 96-hour LC_{50} values for fathead minnows (*Pimephales promelas*, a standard test species) range from 4,000 to 6,600 ppb (Dorn et al 1993; Staples et al 1998; Trumbo 1999). For NP8E, a 96-hour LC_{50} of 4,100 to 5,400 ppb was determined for juvenile rainbow trout (Calamari and Marchetti, 1973). For NP8.9E, a 48-hour LC_{50} of 11,200 to 14,000 ppb was determined for the Japanese medaka (*Oryzias latipes*) (Yoshimura 1986). These acute toxicity values for NP8-9E are at least 1 order of magnitude less than NP. For NP10E acute toxicity, 96-hour LC_{50} values for adult cod (*Gadus moorhua*) and flounder (*Pleuronectes flesus*) range from 2,500 to 6,000 ppb depending upon water temperature (Swedmark *et al*, 1971).

In terms of NP acute toxicity, most 96-hour LC_{50} values for tested fish species range from 100 to 460 ppb (refer to Table 2). The lowest tested 96-hour LC_{50} was for the salt-water species flounder (*Pleuronectes americanus*), with a value of 17 ppb (Lussier et al 2000). In fathead minnow, the 96-hour LC_{50} value for NP ranges from 128 to 320 ppb (Naylor, 1995; Sappington et al 2001; Servos 1999). Rainbow trout (*Oncorhynchus mykiss*) appear similar to fathead minnow with an NP 96-hour LC_{50} of 190 to 270 ppb (Naylor, 1995; Sappington et al 2001; Servos 1999; US EPA 1996). Atlantic salmon (*Salmo solar*) have been tested against NP with 96-hour LC_{50} results in the range of 130 to 900 ppb (McLeese et al 1981, 1980). In the Japanese medaka, the 48-hour LC50 for NP is 1,400 ppb (Yoshimura 1986). Sheepshead minnow appear somewhat more tolerant of NP than fathead minnow, with a 96-hour LC_{50} of 460 ppb (Sappington et al 2001).

In an interesting study used to compare the acute toxicity of NP in surrogate species against threatened or endangered fish species, it was found that the Apache trout, greenback cutthroat trout, and Lahontan trout all had very similar results to the rainbow trout surrogate (96-hour LC_{50} values of 150 to 180 ppb as compared to the rainbow trout 190 ppb) (Sappington et al 2001). Correlations were not as good between warm water threatened or endangered fish, such as the bonytail chub, Colorado pikeminnow, and razorback sucker, and the surrogate species the fathead minnow (96-hour LC_{50} values of 170 to 290 ppb as compared to 270 ppb) (*ibid*). However, the authors conclude that a 2X safety factor should be sufficient to provide a conservative estimate for listed cold and warm freshwater fish species (*ibid*).

The acute toxicity of the environmental metabolite NP1EC to fathead minnows indicates a 96-hour EC_{50} of 2,000 ppb (Staples et al 1998). In a study of Japanese medaka or killifish, a 48-hour EC_{50} for NP1EC was determined to be 9,600 ppb; for NP2EC, the 48-hour EC_{50} was 8,900 ppb (Yoshimura 1986).

It would appear that in terms of acute toxicity to fish, NP9E is of relatively low acute toxicity, as are the likely environmental metabolites that would be found in water (the NPECs). The NPECs would appear to be slightly more acutely toxic to fish than NP9E. NP is an order of magnitude more toxic to fish than the NP9E or NPECs.

In terms of sub-chronic and chronic toxicity, there is little data on NPEs. In one study, a 7-day No Observable Effect Concentration (NOEC) for NP9E was determined to be 1,000 ppb on fathead minnows based on growth (Dorn et al, 1993). In a 42-day test, fathead minnows were exposed to NP9E at rates up to 5.5 ppb with no mortality and no effects to secondary sex characteristics (Miles-Richardson et al 1999). In a 14-day test, rainbow trout were exposed to NP8E with a resultant LC_{50} of 4,250 ppb (Calamari and Marchetti, 1973).

For NP, the subchronic NOEC varies with species, but the lab-determined values range from 1-23 ppb (28- to 90-day values) (Schwaiger et al 2000; Servos 1999; Staples et al 1998). Liber et al, 1999b evaluated effects on bluegills in a littoral enclosure experiment at mean maximum concentrations of 5,

23, 76, and 243 ppb over 20 days. They found that survival after 70 days was reduced at 243 ppb, while effects to behavior were seen at 76 and 243 ppb (ibid). The drop in survival could have been due to effects to the prey base. A NOEC from Liber et al 1999b would be 76 ppb as a mean maximum, or 45 ppb as an average over the 20 days. Giesy et al 2000 determined a NOEC of > 3.4 ppb for fathead minnow based on a 7-day exposure to NP during a 42-day evaluation of fecundity. In a 42-day test, fathead minnows were exposed to NP at rates up to 3.4 ppb with no mortality and no effects to secondary sex characteristics (Miles-Richardson et al 1999). In a study in rainbow trout, Ashfield et al 1998 exposed female trout from day of hatch for 22 or 35 days to NP, NP2E and NP1EC at 0, 1, 10, 30, or 50 ppb, and monitored effects for 108 or 466 days. NP had effects to growth at 30 ppb (body weight, length) and to ovosomatic index (*ibid*). There is some indication that exposure to NP (10-40 days at 1 and 10 ppb) may have some minor effects on the chemistry of skin mucous of trout (Burkhardt-Holm et al 2000). In a unique epidemiological study that looked at Atlantic salmon fisheries that had been exposed to aminocarb (an insecticide) and NP (as a surfactant) in the mid 1970's through mid 1980's, there was a negative correlation between returns of salmon and the percentage of the watershed treated with the mixture (Fairchild et al 1999). Based on water samples of aminocarb, the authors assume that levels of NP in these creeks reached 20 ppb, with levels >800 ppb in stagnant waters.

In another study on rainbow trout, Jobling et al determined that testicular growth was completely inhibited at 54 ppb (Staples et al 1998). In a recent study of Chinook salmon, alevins exposed to NP at rates up to 10 ppb for 29 days post-hatch showed no effects to sexual differentiation (genetic sex matched the gonadal sex) when evaluated at 103 days post-hatch (Afonso et al 2003). In this same study, exposure to high amounts of bleached kraft mill effluent, as well as primary and secondary sewage treatment effluent did show some gonadal effects in male alevins. These effluents did contain varying amounts of NP and NPEs as well as other potential estrogenic compounds, so effects as a result of additive doses were possible. In a study in adult male medaka exposed to 100 ppb of NP over 6 weeks, there was an increase in apoptosis in spermatocytes, Sertoli cells, and Leydig-homologue cells, but not in spermatids, intestine, liver, or kidneys (Weber et al 2002). The authors conclude that these effects to the testes would likely lead to a decrease in the number and quality of sperm with prolonged NP exposure (*ibid*). In another study in medaka, males were exposed to NP for two weeks at approximately 7, 22, and 66 ppb, and then allowed to mate with unexposed females (Shioda and Wakabayashi 2000). Although not statistically significant, there was a decrease in hatching success of eggs at the highest dose as compared to controls (*ibid*).

As concerns the environmental metabolites: in the study by Ashfield et al 1998, exposure to NP1EC at rates up to 50 ppb for 35 days after hatch in rainbow trout had no dose-dependent effects on growth or ovosomatic index as measured after 108 or 466 days. In an unpublished study by Williams, 1997 (cited in Environment Canada 2001, Table 9), with fathead minnows, a NOEC of 1000 ppb was established for NP1EC.

Sublethal effects from exposure to NP10E in codfish (*Gadus morhua*) have been demonstrated in studies by Swedmark (Swedmark *et al* 1971, Swedmark 1976 as referenced in Lewis 1991). Avoidance responses were seen at rates of >1 mg/L (1,000 ppb). This exposure rate was 3 orders of magnitude higher than needed to elicit the same response from NP (2 µg/L or 2 ppb) in the same species. Swedmark notes that sublethal effects appear as impaired locomotor activity and breathing rate, and it appeared that the surfactant absorbed to the gill epithelium and interfered with gas diffusion (Swedmark et al 1971). The effects on swimming and breathing at >1 mg/L (1,000 ppb) suggest a narcotic effect, with these effects remaining reversible over a long period (*ibid*). In another study listed in Lewis 1991,

Hoglund (1976) demonstrated an avoidance response in the same species with the same material at a much lower rate (2 µg/L or 2 ppb), and at the same rate as a similar response by NP. This result from Hoglund would seem to be inconsistent with the known relative toxicities of these two compounds.

Bioconcentration potential of the short-chain ethoxylates (NP, NP1E, NP2E) in freshwater fish and other aquatic biota appears to be low to moderate ranging up to about 740 (Ahel et al 1993; Liber et al 1999b; Snyder et al 2001; US EPA 1996). Little data exists on the bioconcentration of longer chain NPEs, but based on their structure they are not expected to bioaccumulate (Environment Canada 2001a, Servos 1999).

There are a few studies in the literature on the metabolism of NPE and NP in fish. In a study by Cravedi et al, 2001, after a single oral dose of NP2E (10 mg/kg wet weight, gavage using a gelatin capsule) to rainbow trout, most of the NP2E excretion was through the bile, consisting mostly of the glucuronide conjugate of NP2E. There were no detections of NP or the NP- glucuronides, indicating that NP is not an *in vivo* metabolite of NP2E in rainbow trout. Whole body residue analysis showed extensive distribution through the body; the NP2E or metabolites were not preferentially found in fat. The authors surmise that this is due to a rapid and extensive biotransformation of NP2E to more polar metabolites (*ibid*). In Liber et al, 1999a, after 20 days of exposure in a littoral enclosure to NP, depuration of NP in bluegills was rapid, with nondetectability in tissues in fish collected within 6 days of cessation of treatments at an average of 3 ppb, and within 28-34 days after cessation at an average of 14 ppb.

In Coldham et al 1998, a single intravenous dose of NP was given to juvenile trout (0.375 mg, ~3 mg/kg). This was not meant to represent a realistic dose or method of exposure, only to be able to track metabolites. Relative concentrations of NP: bile>>feces>>liver>pyloric caecae>kidney>rest of body. The elimination half-life in muscles was determined to be 99 hours. Despite rapid metabolism and excretion, a substantial depot of parent compound remained in muscle, especially near site of injection, which could have implications for the maintenance of NP residues and associated biological activity. However, this study involved a mode of exposure that would not be expected in nature. In another NP metabolism study by Lewis and Lech 1996, fish were statically exposed to 18 or 36 ppb in water. NP or its metabolites were distributed throughout the body, including edible tissues of dorsal muscle and fat. This study determined a half-life of 19-20 hours in the muscle and fat. Bioaccumulation factor in viscera and carcass for NP ranged from 24 in carcass to 98 in viscera. The authors conclude that NP appears to be metabolized through oxidation and glucuronic acid formation and excreted in bile, although the identity of the specific metabolites was not confirmed (*ibid*). In an experiment on juvenile Atlantic salmon, Arukwe et al demonstrated that NP was metabolized and excreted as a glucuronide and related hydroxylated compounds; there were no traces of the parent compound in bile or urine (Arukwe et al 2000a and 2000b). The authors determined that two routes of clearance were utilized after aqueous exposure, primarily the biliary system followed by the urinary system. Half-life of metabolites in the carcass, viscera, and muscle was between 24 and 48 hours. When exposure was by aqueous suspension, the localization of NP was restricted to skin and surficial epithelium of the oral cavity and the gills; after immersion in clean water for 48 hours, this surface pattern was absent (*ibid*).

4.1.3.2. - Frogs and Toads (Refer also to Table 3 in Appendix 3)

Research on effects of NP and NPE on frog and toad species is more limited then with fish and the available research has involved only the embryo or tadpole stages of development.

In two recent studies involving NP8E, Mann and Bidwell tested embryos of three species and tadpoles of six species including several indigenous Australian species (Mann and Bidwell 2000, 2001). In the embryo study, LC_{50} values ranged from 3.9 to 9.2 ppm (time periods of 96 to 140 hours), with the common test species *Xenopus laevis* being the most sensitive. These LC_{50} values are comparable to values for freshwater fish. Developmental EC_{50} values ranged from 2.8 to 8.8 ppm, while the minimum concentration inhibiting growth (an LOEC) ranged from 1 to 4 ppm. The tadpole study involved determining when mild or full narcosis occurred. Mild narcosis EC_{50} values ranged from 2.3 to <10.6 ppm. Water temperature increases did not affect this, but reduced dissolved oxygen in water resulted in EC_{50} values reduced by about half as compared to normal levels of oxygen. The authors observed that the tadpoles exhibited recovery from narcosis during the life of the test, indicating some mechanism of adaptation.

For NP, acute toxicity values for amphibians ranged from 75 to 120 ppb in water (96-hour to 14-day LC_{50}) (Dwyer et al 1997, Weeks et al 1996, both cited in Servos, 1999) and 260 mg/kg in 10 to 30 day LC_{50} values for dosed sediments (Naylor 1995; Ward and Boeri, 1992 as cited in Servos 1999; US EPA 1996). Using *Xenopus laevis*, Fort and Stover 1997 determined a 14-day NOEC for tail resorption of 25 ppb exposure to NP. Kloas et al 1999 showed that NP exposure for 12 weeks to *X. laevis* tadpoles at 22 ppb caused a significant increase in the percentage of female frogs; this effect was not seen at 2.2 ppb.

4.1.3.3. - Other Aquatic Organisms (refer also to Table 3 in Appendix 3)

For NP9E, the toxicity to aquatic invertebrates is reduced from NP, demonstrating the same relationship as is found in fish and amphibians. For the common lab test species *Daphnia magna*, the 48-hour EC_{50} has been derived as 14,000 ppb (Kravetz et al, 1991; Dorn et al 1993). In two subchronic studies, a *Daphnia* 7-day NOEC (growth) value of 10,000 ppb for NP9E is derived (Dorn et al, 1993; Staples et al 1998). For mysid shrimp, the 48-hour LC_{50} value for exposure to NP9E ranges from 900 – 2,000 ppb (Environment Canada 2001a; Hall et al, 1989; Patoczka and Pulliam, 1990).

One study looked at exposure of daphnia to NP2E and NP2EC, and derived a 48-hour LC_{50} of 115 to 198 ppb for NP2E and 770 to 1,295 ppb for NP2EC (Maki 1998).

In Swedmark et al 1971, sublethal effects to mussels, cockles, and barnacles after exposure to NP10E were seen at 2-5 mg/L (ppm); effects to locomotion of a decapod, hermit crab and shore crab were seen at 20-40 mg/L (ppm)(Swedmark et al 1971, Lewis 1991).

Henry et al 1994 conducted a study with X-77, an NPE-based surfactant, mixed with the Rodeo formulation of glyphosate and applied to freshwater wetlands as well as in lab acute toxicity tests to determine toxic effects to invertebrates. 48- and 96-hour LC_{50} values for X-77 ranged from 2.0 to 14.1 mg/L for 4 species of invertebrates. This was about 2 orders of magnitude greater acute toxicity than Rodeo. Mortality patterns in treated and untreated wetlands were similar, indicating a lack of acute toxicity from the operational application of the tank mix. Based on application rates applied, and assuming X-77 would be detected at same rate as it was applied, the authors concluded that the margin of safety would indicate no acute risk. However they concede that little is known about any potential chronic effects to aquatic organisms from this type of application.

Various species of freshwater and marine invertebrates have been tested against NP. 96-hour LC_{50} values range from about 20 to about 775 ppb (Lussier et al 2000; Staples et al 1998; Hecht, Boese 2002; Servos 1999; Comber et al 1993; Bechmann 1999; McLeese et al 1980, 1981). The LC_{50} for NP and

NP2E are similar for Daphnia (Servos 1999). In terms of chronic toxicity, a 28-day NOEC for mysid shrimp growth after exposure to NP is 4 ppb (Naylor, 1995; Servos 1999; Staples et al 1998). Daphnia have a slightly higher 21-day NOEC (reproduction) of 24 and 116 ppb (Comber et al 1993; Brooke, 1993 as cited in Servos 1999). Daphnia embryotoxicity threshold occurs at 44 ppb (LeBlanc et al 2000). The marine copepod, *Tisbe battagliai*, had a 53-day NOEC of 20 ppb (Bechmann 1999). In littoral enclosure studies, there were no effects seen on macroinvertebrates at levels of NP up to 23 ppb; no effects to zooplankton were seen at levels of NP exposure of 5 ppb (O'Halloran et al, 1999; Schmude et al, 1999).

In a study by Baldwin et al, 1997, adult Daphnia magna were exposed to NP for 48 hours in an acute exposure regime, while in a chronic exposure test, 24-hour old Daphnia magna were exposed to various levels of NP over a 3-week period. The study was intended to look at the effects of NP exposure on the ability of the females to metabolize testosterone; inhibition of this process can result in a buildup of testosterone in females, affecting reproductive success. The acute exposure portion of the study found that 48-hour exposure to 100 ppb NP (but not 25 or 50 ppb) caused an accumulation in testosterone. In the chronic exposure portion of the study, there were no effects on survival or fecundity in the F0 or F1 generations at 6 or 25 ppb. Survival was not affected at levels up to 100 ppb in the F0 generation, but was affected at 50 ppb in the F1 generation. Fecundity in the F0 generation was affected at 50 and 100 ppb, but only significantly different from controls at 100 ppb. A chronic effect concentration for steroid metabolism is displayed as 25 ppb in the study, however the data only shows a statistically significant effect at 100 ppb.

In a study of NP applied to outdoor microcosms at that achieved maximum average concentrations of 5, 23, 76, and 243 µg/L (O'Halloran et al 1999) there were significant declines in zooplankton abundance and insect emergence only at the highest NP concentration of 243 µg/L, although there were sensitive taxa affected at 23 µg/L. Although some sensitive taxa were affected at 23 µg/L, in terms of abundance, the overall zooplankton community structure was relatively unaffected (as measured using the Shannon-Wiener diversity index). Reductions in abundance seemed to be linked to feeding habits, with water filter feeders less affected than those zooplankton that fed on periphyton that likely had residual NP on the surface. The authors conclude that a Maximum Acceptable Toxicant Concentration (MATC) to protect zooplankton is approximately 10 µg/L (*ibid*). In a study involving aquatic hermaphrodite snails exposed to NP at 1, 10 or 100 µg/L for 7 weeks, there were no histopathological changes at any concentration, no effects to mortality or growth, no effects to fecundity or hatchability of F1 eggs (Czech et al, 2001). When these same species of snail were exposed to 100 µg/L for 12 weeks, there were effects to the epithelial tissue of foot and lung, and skin inflammation, as well as a drop in fecundity (*ibid*). No other doses were tested for the 12-week period.

Exposure to NP9E results in NOEC (growth) values of 8,000 ppb for green algae and a 96-hour EC_{50} (growth) of 12,000 ppb (Naylor, 1995; Dorn et al 1993). For NP, the most sensitive aquatic plant species tested has been a marine alga, with a 96-hour EC_{50} (growth) of 27 ppb, and a NOEC of 10 ppb (Naylor, 1995). Green algae and duckweed have NOEC (growth) values ranging from 90 to 900 ppb after 96-hour exposure to NP (Environment Canada 2001a; Naylor, 1995; Staples et al 1998). Duckweed seems to be more tolerant of NP than the algae. In a littoral enclosure study of exposure to NP, there were no effects to aquatic macrophytes (*Chara* and *Potamogeton*) while there was a small increase in periphyton biomass at the highest mean average concentration of 243 µg/L over 20 days (Liber et al 1999a).

4.2 Exposure Assessment

4.2.1. Overview

Terrestrial animals might be exposed to any applied herbicide and surfactant mixture from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. In acute exposure scenarios, the highest exposures for small terrestrial vertebrates will occur after a direct spray and could reach up to about 40 mg/kg under typical exposure conditions (assuming 100% absorption) and up to about 160 mg/kg under more extreme conditions. Doses from the consumption of contaminated vegetation are about 2.1 mg/kg under typical conditions for a small mammal with an upper range of 320 mg/kg for a large mammal. The consumption of contaminated water will generally lead to much lower levels of exposure. A similar pattern is seen for chronic exposures. Based on general relationships of body size to body volume, larger vertebrates will be exposed to lower doses and smaller animals, such as insects, to much higher doses than small vertebrates under comparable exposure conditions. Because of the apparently low toxicity of NP9E to animals, the rather substantial variations in the different exposure assessments have little impact on the assessment of risk to terrestrial animals.

Exposures to aquatic plants and animals are based on essentially the same information used to assess the exposure to terrestrial species from contaminated water. The estimated rate of contamination of water associated with the normal application of NP9E is 0.0125 (0.0031 to 0.0312) mg a.e./L at an application rate of 1 %. For chronic exposure scenarios, the background levels of NP1-2EC of 0.007 (0 to 0.014) mg/L are used. For acute exposure scenarios, the highest estimated concentration of NP9E in water after an accidental spill is about 6.1 mg a.e./L with a range of about 3.0 to 15.1 mg a.e./L.

4.2.2. Terrestrial Organisms

Terrestrial animals might be exposed to any applied NP9E-based surfactant from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation.

For the exposure assessments, general allometric relationships are used to model exposure. In the biological sciences, allometry is the study of the relationship of body size or mass to various anatomical, physiological, or pharmacological parameters. These relationships dictate that, for a fixed level of exposure (e.g., levels of a chemical in food or water), small animals will receive a higher dose, in terms of mg/kg body weight, than large animals will receive. In this risk assessment, generic estimates of exposure are given for a small and large mammal. A body weight of 20 g is used for a small animal, which approximates the body weight of small mammals such as mice, voles, shrews, and bats. For a large mammal, a body weight of 70 kg approximates the body weight of deer.

The exposure assessments for terrestrial animals are summarized in Table 4-1. As with the human health exposure assessment, the computational details for each exposure assessment presented in this section are provided in the worksheets in Appendix 4 to this risk assessment.

4.2.2.1 Direct Spray

In the broadcast application of any herbicide, wildlife species may be sprayed directly. This scenario is similar to the accidental exposure scenarios for the general public discussed in section 3.2.3.2. In a scenario involving exposure to direct spray, the extent of dermal contact depends on the application rate,

the surface area of the organism, and the rate of absorption.

For this risk assessment, two direct spray exposure assessments are conducted. The first involves a 20 g mammal that is sprayed directly over one half of the body surface as the chemical is being applied. The range of application rates as well as the typical application rate is used to define the amount deposited on the organism. The absorbed dose over the first day (i.e., a 24-hour period) is estimated using the assumption of first-order dermal absorption. An empirical relationship between body weight and surface area is used to estimate the surface area of the animal. The estimates of absorbed doses in this scenario may bracket plausible levels of exposure for small mammals based on uncertainties in the dermal absorption rate of NP9E.

Other, perhaps more substantial, uncertainties affect the estimates for absorbed dose. For example, the estimate based on first-order dermal absorption does not consider fugitive losses from the surface of the animal and may overestimate the absorbed dose. Conversely, some animals, particularly birds and mammals, groom frequently, and grooming may contribute to the total absorbed dose by direct ingestion of the compound residing on fur or feathers. Furthermore, other vertebrates, particularly amphibians, may have skin that is far more permeable than the skin of most mammals. Quantitative methods for considering the effects of grooming or increased dermal permeability are not available. As a conservative upper limit, the second exposure scenario is developed in which complete absorption over day 1 of exposure is assumed.

4.2.2.2. Indirect Contact

As in the human health risk assessment (see section 3.2.3.2), the only approach for estimating the potential significance of indirect dermal contact is to assume a relationship between the application rate and dislodgeable foliar residue. The study by Harris and Solomon 1992 (as referenced in SERA 1999) is used to estimate that the dislodgeable residue will be approximately 10 times less than the nominal application rate.

Unlike the human health risk assessment in which transfer rates for humans are available, there are no transfer rates available for wildlife species. As discussed in Durkin et al 1995, the transfer rates for humans are based on brief (e.g., 0.5- to 1-hour) exposures that measure the transfer from contaminated vegetation to uncontaminated skin. Wildlife, compared with humans, is likely to spend longer periods of time in contact with contaminated vegetation.

It is reasonable to assume that for prolonged exposures a steady-state may be reached between levels on the skin, rates of absorption, and levels on contaminated vegetation, although there are no data regarding the kinetics of such a process. The bioconcentration data on NP9E (section 3.2.3.4) as well as the estimated rates of dermal absorption in humans (Section 3.1.7) suggest that NP9E is not likely to preferentially partition from the surface of contaminated vegetation to the surface of skin, feathers, or fur. Thus, a plausible partition coefficient is unity (i.e., the concentration of the chemical on the surface of the animal will be equal to the dislodgeable residue on the vegetation).

Under these assumptions, the absorbed dose resulting from contact with contaminated vegetation will be one-tenth that associated with comparable direct spray scenarios. As discussed in the risk characterization for ecological effects (section 4.4), the direct spray scenarios, assuming first order absorption, result in exposure levels generally below the estimated NOAEL - i.e., hazard quotients below one. Consequently, details of the indirect exposure scenarios for contaminated vegetation are not

further elaborated in this document.

One concern that has been expressed is the potential for surfactants increasing the movement of other harmful materials, such as pesticides, into soils. In a study by Beigel et al, 1998, levels of nonionic NPE-based surfactants at concentrations below 1000 mg/L caused little or no decrease in sorption of a fungicide. At 10,000 mg/L, an increase in sorption was seen (*ibid*). Reported desorption of contaminants is a result of exceptionally high application rates (one study referenced used 100,000-200,000 mg/L) (*ibid*). [In contrast, given fairly conservative rainfall figures of 2 inches for dilution, rates of application in this risk assessment would result in NP9E concentrations of 3.7 mg/L (typical concentration) up to 15 mg/L at the highest concentration.] The increase in sorption seen at low levels is postulated two ways: 1) an increase in soil organic carbon content as a result of surfactant addition created increased affinity for bonding: 2) at low levels, the surfactant sorbs to soil through hydrophobic interactions, leaving the hydrophilic heads that would extend into soil solution, increasing desorption. As more surfactant is added, a bilayer of surfactant is created on soil, with hydrophobic tails of second layer sticking into solution, creating an affinity for additional sorption (*ibid*).

4.2.2.3. Ingestion of Contaminated Vegetation

For this component of the exposure assessment, the estimated amounts of residue on food are based on the relationship between application rate and residue rates on leaves and leafy vegetables. Allometric relationships and species specific data suggest that the amount of food consumed per day by a small mammal (i.e., an animal weighing approximately 20 g) is equal to about 15% of the mammal's total body weight. All of the estimates of ingested dose are based on the assumption that 100% of the diet is contaminated. This is more likely with small animals with a limited range; conversely it is unlikely to occur with large grazing animals like deer or elk. Under the assumption that only 10% of the diet is contaminated, the dose estimates decrease by a factor of 10.

For estimating the effects of longer-term exposures, time-weighted average concentrations are used, which is similar to the approach taken in the human health risk assessment and using the same estimates of foliar halftime as were used in the corresponding human health risk assessment. Also, the longer-term exposure scenario is based on a 90-day post-spray period and uses the geometric mean over this period as the central estimate of the exposed dose, as in the human health risk assessment. Like the acute exposure scenario, this exposure scenario assumes that 100% of the diet is contaminated.

4.2.2.4. Ingestion of Contaminated Water

Estimated concentrations of NP9E in water are identical to those used in the human health risk assessment. The only major differences involve the weight of the animal and the amount of water consumed. There are well-established relationships between body weight and water consumption across a wide range of mammalian species. Mice, weighing about 0.02 kg, consume approximately 0.005 L of water/day (i.e., 0.25 L/kg body weight/day). These values are used in the exposure assessment for the small (20g) mammal. Unlike the human health risk assessment, estimates of the variability of water consumption are not available. Thus, for the acute scenario, the only factors affecting the variability of the ingested dose estimates include the field dilution rates (i.e., the concentration of the chemical in the solution that is spilled) and the amount of solution that is spilled. As in the acute exposure scenario for the human health risk assessment, the amount of the spilled solution is taken as 200 gallons. In the chronic exposure scenario, the only factor that affects the variability is the water contamination rate, (see section 3.2.3.3.2).

Table 4-1: Summary of Exposure Scenarios for Terrestrial Animals

Scenario	Dose (mg/kg/day)		
	Typical	Lower	Upper
Direct spray, small mammal, first order absorption	0.068	0.00097	3.5
Direct spray, small mammal, 100% absorption	40	4	160
Consumption of contaminated veg, small mammal, acute	2.1	0.21	18
Consumption of contaminated veg, large mammal, acute	29	2.9	320
Consumption of contaminated water, spill	0.89	0.44	2.2
Consumption of contaminated water, drift, etc.	0.0018	0.00045	0.0046
Consumption of contaminated veg, small mammal, chronic	0.0034	0.00017	0.057
Consumption of contaminated veg,large mammal, chronic	0.14	0.0046	5.2
Consumption of contaminated water, chronic exp.	0.001	0	0.002

4.2.3. Aquatic Organisms

The potential for effects on aquatic species are based on estimated concentrations of NP9E or NP1-2EC in water that are identical to those used in the human health risk assessment. The estimated rate of contamination of ambient water associated with the normal application of NP9E is 0.0125 mg a.e./L (12.5 ppb). For acute exposure scenarios, the highest estimated concentration of NP9E in water after an accidental spill is about 6.1 mg a.e./L (ppm) with a range of about 3.0 to 15.1 mg a.e./L. As another exposure scenario, if the Forest Service were to overspray an herbicide mixture with an 80% NPE-based surfactant into a small pond or stagnant stream reach, with no foliar interception, instantaneous levels of NP9E could approach 1.5 mg/L (1,500 ppb) and the concentration of NP and the short-chain ethoxylates (NP1E and NP2E) could approach (0.075 mg/L (75 ppb) (refer to worksheet 1 in Appendix 1). Assuming a more realistic live stream, these levels would be quickly lowered as water is mixed through stream flow.

As discussed in section 3.2.3.3, the breakdown of NPE would likely not liberate NP, and any free NP in the surfactant would be broken down in the forested environment or bound to soil particles. Therefore, it is very unlikely that NP would be found in forest streams above the level that might be found in the NP9E mixture originally. As stated in section 4.3, the acute toxicity of NP9E includes this small percentage of NP and short-chain NPEs, so no adjustment for acute exposures is necessary.

Based on environmental fate, the toxicological compound of interest is more likely to be the short chain NPECs (NP1EC, NP2EC), as they will be formed in the forested environment and their persistence would make them more available for aquatic wildlife exposure and for exposure to terrestrial wildlife through water consumption. As stated in section 3.2.3.3.2, the assumed levels of NP1-2EC in water will be based on water monitoring and set at 0.007 mg/L (with a range of 0 to 0.014 mg/L).

4.3 Dose-Response Assessment

4.3.1. Terrestrial Organisms

Based on the discussions in section 3.1, mammalian toxicity is well characterized for NP, less so for NP9E and the carboxylate metabolites. For the chronic exposure scenarios, the 10 mg/kg/day derived NOEL from the NP rat multigeneration study (Nagao et al, 2001) will be used for both NP and NPE. For acute exposure scenarios the lowest 90-day NOEL value of 10 mg/kg/day from the NP9E rat dietary study will be used (Mellon Institute, 1960, as cited in Smyth and Calandra, 1969). This should be considered a conservative value, as the NOEL values from similar tests range up to 40 mg/kg/day, with LOELs beginning at 50 mg/kg/day (refer to Table 3-4).

4.3.2. Aquatic Organisms

As stated in Section 3.2.3.3, although NP is of higher toxicity to aquatic organisms than NPE or NPEC, there is sufficient information in the literature to make the assumption that in a forested environment, contamination of surface water is more likely to involve NP9E in the short-term and NP1-2EC in the long-term. As such, indicators of risk will be based upon these two compounds, not NP.

For NP9E, the value that will be used to establish the aquatic acute no-effect level is the 7-day NOEC (growth) for minnows of 1,000 ppb (Dorn et al, 1993; Staples et al 1998). As the species that have had testing with the longer chain NPEs all have similar values, no interspecies factor will be used. Similar to the human health risk assessment, for acute exposures, this value will be used, with the assumption that acute toxicity tests involving NP9E includes a small percentage of the short-chain ethoxylates, as well as small amounts of NP.

For NP1EC and NP2EC, the NOEC value in fathead minnows, 1,000 ppb (Williams 1997, unpublished, as cited in Environment Canada 2001a) will be used to establish a chronic protective level of 100 ppb by dividing by an interspecies factor of 10.

Based on the limited data in Appendix 3, Table 3, it appears that frogs are similar or somewhat less sensitive than fish species. Levels of exposure that result in low levels of risk to fish should therefore also be similarly protective of frogs.

For aquatic invertebrates, the 7-day NOEC for NP9E of 10 mg/L for *Daphnia* spp. found in Dorn et al, 1993 will be used for acute exposures. For chronic exposures, since no testing has been done using the NP1-2ECs, the 21-day NP NOEC for *Daphnia magna* from Comber et al 1993, will be used (0.024 mg/L).

For aquatic plants, the 96-hour NP9E NOEC (growth) of 8 mg/L for green algae (Dorn et al 1993; Naylor 1995) will be used for acute exposures. There are no chronic exposure studies for aquatic plants.

4.4 Risk Characterization

4.4.1 Terrestrial Organisms

Similar to the discussion of human risk in Section 3.4, the benchmark NOEL values described above were used to derive hazard quotients for each exposure scenario. These Hazard Quotients are a quantitative expression of risk, calculated by dividing the expected dose by the NOEL. Table 4-2 displays the Hazard Quotients for the terrestrial animals.

Based on the Hazard Quotients in Table 4-2, several of the scenarios represent potential risk to terrestrial wildlife. With the typical application rates, two of the acute scenarios result in hazard quotients that exceed unity (direct spray with 100% absorption, and consumption of contaminated vegetation by a large animal). As stated in Section 3.3.3, acute doses from 10 to 40 mg/kg/day may not represent a risk to mammals, in which case these typical scenarios may be of low risk, even though the hazard quotient exceeds unity. As stated previously it is also less likely that a large grazing mammal, such as a deer would feed exclusively in a treated area. At the highest application rates, these same two acute exposures scenarios represent a high risk of effects. At exposures above 250 mg/kg/day (an HQ>25) frank toxic effects are possible. At exposures between 100 and 250 mg/kg/day, as stated in section 3.3.3, effects are uncertain in terms of seriousness, with inconsistent results in the various studies. Both scenarios are unlikely, as discussed previously. Given the assumptions, combined with typical animal behaviors, the actual exposure rate for a directly sprayed small mammal is likely somewhere in between the two scenarios of first order absorption and 100% absorption.

Table 4-2: Summary of Quantitative Risk Characterization for Terrestrial Animals

Scenario	Hazard Quotient		
	Typical	Lower	Upper
Direct spray, small mammal, first order absorption	0.007	0.0001	0.4
Direct spray, small mammal, 100% absorption	4	0.4	16
Consumption of contaminated veg, small mammal, acute	0.2	0.02	2
Consumption of contaminated veg, large mammal, acute	3	0.3	32
Consumption of contaminated water, spill	0.09	0.04	0.2
Consumption of contaminated water, drift, etc.	0.0002	0.00004	0.0005
Consumption of contaminated veg, small mammal, chronic	0.0003	0.00002	0.006
Consumption of contaminated veg, large mammal, chronic	0.01	0.0005	0.5
Consumption of contaminated water, chronic exp.	0.0001	0	0.0002

4.4.2. Aquatic Organisms

The duration of an exposure must be considered, which, in the case of aquatic environments in the National Forests, would be short; the compounds of concern are broken down and their concentration reduced through dilution, as well as binding of the compounds to stream sediments.

The ambient levels of NP9E (including a small percentage of NP and NP1-2E) assumed to be present from normal operations (12.5 ppb with a range of 3.1 to 31.2 ppb) would be protective of all aquatic organisms at all application rates. For fish, these assumed levels are at least 30 times lower than the

1,000 ppb protective level for NP9E. For aquatic invertebrates, exposure levels are at least 320 times lower than the 10,000 ppb protective level for NP9E. Given the chronic exposure to NP1-2EC of 7 ppb (0 to 14 ppb range), there should be no chronic toxic risk to aquatic species. Refer to worksheet G03 in Appendix 4. There is little potential for increased vitellogenin levels in fish at both acute and chronic exposure levels.

Both the overspray and the spill scenarios involve levels of NP9E that could represent a risk of toxic effects. The overspray scenario exceeds the acute NP9E threshold for fish by a factor of 1.5 (typical rate), up to a factor of 4.9 (highest rate). The overspray scenario should not represent an acute risk to aquatic invertebrates. With a spill, the NP9E threshold for acute effects to fish is exceeded by a factor of 6.1 (central estimate), up to a factor of 15.1 (highest rate), while for aquatic invertebrates, the threshold for acute effects is exceeded at the highest concentration rate, by a factor of 1.5 (Refer to Worksheet D05). Aquatic plants would have values intermediate between fish and invertebrates. In a stagnant small pond or stream reach, there could be effects seen to aquatic organisms. In a live stream, the more realistic scenario would be a short-term pulse of concentrated NP9E moving downstream, mixing with water and being broken down into NP1-2EC and/or partitioning into sediments. The effects of a short pulse should be minor on aquatic organisms as the short exposure time would result in lower doses than are discussed here.

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Glossary

Absorption -- The process by which the agent is able to pass through the body membranes and enter the bloodstream. The main routes by which toxic agents are absorbed are the gastrointestinal tract, lungs, and skin.

Acute exposure -- A single exposure or multiple exposures occurring within a short time (24 hours or less).

Additive effect -- A situation in which the combined effects of two chemicals is equal to the sum of the effect of each chemical given alone. The effect most commonly observed when two chemicals are given together is an additive effect.

Adjuvant(s) -- Formulation factors used to enhance the pharmacological or toxic agent effect of the active ingredient.

Adsorption -- The tendency of one chemical to adhere to another material.

Adverse-effect level (AEL) -- Signs of toxicity that must be detected by invasive methods, external monitoring devices, or prolonged systematic observations. Symptoms that are not accompanied by grossly observable signs of toxicity. In contrast to Frank-effect level.

Allometric -- Pertaining to allometry, the study and measure of growth. In toxicology, the study of the relationship of body size to various physiological, pharmacological, pharmacokinetic, or toxicodynamic processes among species.

Assay -- A kind of test (noun); to test (verb).

Bioconcentration factor (BCF) -- The concentration of a compound in an aquatic organism divided by the concentration in the ambient water of the organism.

Biologically sensitive -- A term used to identify a group of individuals who, because of their developmental stage or some other biological condition, are more susceptible than the general population to a chemical or biological agent in the environment.

Cancer potency parameter -- A model-dependent measure of cancer potency $(mg/kg/day)^{-1}$ over lifetime exposure. [Often expressed as a_{q1} * which is the upper 95% confidence limit of the first dose coefficient (q1) from the multistage model.]

Carcinogen -- A chemical capable of inducing cancer.

Carcinoma -- A malignant tumor.

Carrier -- In commercial formulations of insecticides or control agents, a substance added to the formulation to make it easier to handle or apply.

Chronic exposure -- Long-term exposure studies often used to determine the carcinogenic potential of chemicals. These studies are usually performed in rats, mice, or dogs and extend over the average lifetime of the species (for a rat, exposure is 2 years).

Connected actions -- Exposure to other chemical and biological agents in addition to exposure to the control agent during program activities to control vegetation.

Contaminants -- For chemicals, impurities present in a commercial grade chemical. For biological agents, other agents that may be present in a commercial product.

Controls -- In toxicology or epidemiological studies, a population that is not exposed to the potentially toxic agent under study.

Cumulative exposures -- Exposures that may last for several days to several months or exposures resulting from program activities that are repeated more than once during a year or for several consecutive years.

Dams – A term used to designate females of some species such as rats.

Degraded -- Broken down or destroyed.

Dermal -- Pertaining to the skin.

Dermatitis: Inflammation of the skin, either due to direct contact with an irritating substance, or to an allergic reaction.

Dislodgeable residues – The residue of a chemical or biological agent on foliage as a result of aerial or ground spray applications, which can be removed readily from the foliage by washing, rubbing or having some other form of direct contact with the treated vegetation.

Dose-response assessment -- A description of the relationship between the dose of a chemical and the incidence of occurrence or intensity of an effect.

Drift -- That portion of a sprayed chemical that is moved by wind off a target site.

EC_{50} -- A concentration that causes 50% inhibition or reduction.

Empirical -- Refers to an observed, but not necessarily fully understood, relationship in contrast to a hypothesized or theoretical relationship.

Endocrine – the system in the body consisting of organs that generate compounds that are transported elsewhere in the body and used for regulation of some other part of the body. Examples are the thyroid, the adrenals, and the pituitary glands.

Endogenous – Growing or developing from or on the inside.

Enzymes -- A biological catalyst; a protein, produced by an organism itself, that enables the splitting (as in digestion) or fusion of other chemicals.

Epidemiological study -- A study of a population or human populations. In toxicology, a study that examines the relationship of exposures to one or more potentially toxic agent to adverse health effects normally in human populations.

Estrogen - Any of several steroid hormones produced chiefly by the ovaries and responsible for promoting estrus and the development and maintenance of female secondary sex characteristics.

Exposure assessment -- The process of estimating the extent to which a population will come into contact with a chemical or biological agent.

Extrapolation -- The use of a model to make estimates outside of the observable range.

Fetal anomaly – An abnormal condition in a fetus, which is usually the result of a congenital defect.

Formulation -- A commercial preparation of a chemical including any inerts or contaminants.

Frank effects -- Obvious signs of toxicity.

Frank-effect level (FEL) -- The dose or concentration of a chemical or biological agent that causes gross and immediately observable signs of toxicity.

Gavage -- The placement of a toxic agent directly into the stomach of an animal, using a gastric tube.

Genotoxic -- Causing direct damage to genetic material. Associated with carcinogenicity.

Geometric mean -- The measure of an average value often applied to numbers for which a log normal distribution is assumed.

Gestation -- The period between conception and birth; in humans, the period known as pregnancy.

Half-time or half-life -- For compounds that are eliminated by first-order kinetics, the time required for the concentration of the chemical to decrease by one-half.

Hazard quotient (HQ) -- The ratio of the estimated level of exposure to the RfD or some other index of acceptable exposure.

Hazard identification -- The process of identifying the array of potential effects that an agent may induce in an exposed human population.

Hematological -- Pertaining to the blood.

Hematology -- One or more measurements regarding the state or quality of the blood.

Henry's law constant -- An index of the tendency of a compound to volatilize from aqueous solutions.

Herbicide -- A chemical used to control, suppress, or kill plants, or to severely interrupt their normal growth processes.

Histopathology -- Signs of tissue damage that can be observed only by microscopic examination.

Hydrolysis -- Decomposition or alteration of a chemical substance by water.

Hydroxylation -- The addition of a hydrogen-oxygen or hydroxy (-OH) group to one of the rings. Hydroxylation increases the water solubility of aromatic compounds. Particularly when followed by conjugation with other water-soluble compounds in the body, such as sugars or amino acids, hydroxylation greatly facilitates the elimination of the compound in the urine or bile.

Hyperplasia – An abnormal increase in the number of cells composing a tissue or organ.

In vivo -- Occurring in the living organism.

In vitro -- Isolated from the living organism and artificially maintained, as in a test tube.

Inerts -- Adjuvants or additives in commercial formulations of pesticides that are not readily active with the other components of the mixture.

Interpolation -- The use of mathematical models within the range of observations

Intraperitoneal -- Injection into the abdominal cavity.

Invertebrate -- An animal that does not have a spine (backbone).

Irritant effect -- A reversible effect, compared with a corrosive effect.

LC_{50} (lethal concentration$_{50}$) -- A calculated concentration of a chemical in air or water to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

LD_{50} (lethal dose$_{50}$) -- The dose of a chemical calculated to cause death in 50% of a defined experimental animal population over a specified observation period. The observation period is typically 14 days.

Lowest-observed-adverse-effect level (LOAEL) -- The lowest dose of a chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphatic – Pertaining to lymph, a lymph vessel, or a lymph node.

Lymph – A clear water fluid containing white blood cells. Lymph circulates throughout the lymphatic system, removing bacteria and certain proteins from body tissue. It also is responsible for transporting fat from the small intestine and supplying mature lymphocytes to the blood.

Malignant -- Cancerous.

Margin of safety (MOS) -- The ratio between an effect or no effect level in an animal and the estimated human dose.

Metabolite -- A compound formed as a result of the metabolism or biochemical change of another compound.

Metameter -- Literally, the unit of measure. Used in dose-response or exposure assessments to describe the most relevant way of expressing dose or exposure.

Microorganisms -- A generic term for all organisms consisting only of a single cell, such as bacteria, viruses, and fungi.

Microsomal -- Pertaining to portions of cell preparations commonly associated with the oxidative metabolism of chemicals.

Minimal risk level (MRL) -- A route-specific (oral or inhalation) and duration- specific estimate of an exposure level that is not likely to be associated with adverse effects in the general population, including sensitive subgroups.

Mitochondria – Sub-cellular organelles involved in the conversion of food to stored chemical energy.

Most sensitive effect -- The adverse effect observed at the lowest dose level, given the available data. This is an important concept in risk assessment because, by definition, if the most sensitive effect is prevented, no other effects will develop. Thus, RfDs and other similar values are normally based on doses at which the most sensitive effect is not likely to develop.

Multiple chemical sensitivity -- A syndrome that affects individuals who are extremely sensitive to chemicals at extremely low levels of exposure.

Mutagenicity -- The ability to cause genetic damage (that is damage to DNA or RNA). A mutagen is substance that causes mutations. A mutation is change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

Non-target -- Any plant or animal that a treatment inadvertently or unavoidably harms.

No-observed-adverse-effect level (NOAEL) -- The dose of a chemical at which no statistically or biologically significant increases in frequency or severity of adverse effects were observed between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

No-observed-effect level (NOEL) -- The dose of a chemical at which no treatment-related effects were observed.

Normal distribution -- One of several standard patterns used in statistics to describe the way in which variability occurs in populations.

Octanol-water partition coefficient (K_{ow}) -- The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution.

Ocular -- Pertaining to the eye.

Parenteral -- Any form of injection.

Partition -- In chemistry, the process by which a compound or mixture moves between two or more media.

Pathogen – A living organism that causes disease; for example, a fungus or bacteria.

Pathway -- In metabolism, a sequence of metabolic reactions.

Permeability – The property or condition of being permeable. In this risk assessment, dermal permeability refers to the degree to which a chemical or herbicide in contact with the skin is able to penetrate the skin.

pH -- The negative log of the hydrogen ion concentration. A high pH (>7) is alkaline or basic and a low pH (<7) is acidic.

p_{Ka} -- The negative log of the hydrogen ion concentration or pH at which 50% of a weak acid is dissociated.

Pharmacokinetics -- The quantitative study of metabolism (i.e., the processes of absorption, distribution, biotransformation, elimination).

Phytoestrogen - A naturally occurring compound of plants, such as soybeans, or plant products, such as whole grain cereals, that acts like estrogen in the body.

Prospective -- Looking ahead. In epidemiology, referring to a study in which the populations for study are identified prior to exposure to a presumptive toxic agent, in contrast to a retrospective study.

Pup – The offspring or young of various animal species.

Reference dose (RfD) -- Oral dose (mg/kg/day) not likely to be associated with adverse effects over a lifetime exposure, in the general population, including sensitive subgroups.

Reproductive effects -- Adverse effects on the reproductive system that may result from exposure to a chemical or biological agent. The toxicity of the agents may be directed to the reproductive organs or the related endocrine system. The manifestations of these effects may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions dependent on the integrity of this system.

Resorption -- Removal by absorption. Often used in describing the unsuccessful development and subsequent removal of post-implantation embryos.

Retrospective -- Looking behind. In epidemiology, referring to a study in which the populations for study are identified after exposure to a presumptive toxic agent, in contrast to a prospective study.

RfD – See reference dose.

Route of exposure -- The way in which a chemical or biological agent enters the body. Most typical routes include oral (eating or drinking), dermal (contact of the agent with the skin), and inhalation.

Scientific notation -- The method of expressing quantities as the product of a number between 1 and 10 multiplied by 10 raised to some power. For example, in scientific notation, 1 kg = 1,000 g would be expressed as 1 kg = 1 x 10^3 g and 1 mg = 0.001 g would be expressed as 1 mg = 1 x 10^{-3} g .

Sensitive subgroup -- Subpopulations that are much more sensitive than the general public to certain agents in the environment.

Sensitization – A condition in which one is or becomes hypersensitive or reactive to an agent through repeated exposure.

Species-to-species extrapolation -- A method involving the use of exposure data on one species (usually an experimental mammal) to estimate the effects of exposure in another species (usually humans).

Sub-chronic exposure -- An exposure duration that can last for different periods of time, but 90 days is the most common test duration. The sub-chronic study is usually performed in two species (rat and dog) by the route of intended use or exposure.

Substrate -- With reference to enzymes, the chemical that the enzyme acts upon.

Surfactant – A specific type of additive to a pesticide formulation that is intended to reduce the surface tension of the carrier, to allow for greater efficacy of the pesticide.

Synergistic effect -- A situation is which the combined effects of two chemicals is much greater than the sum of the effect of each agent given alone.

Systemic toxicity -- Effects that require absorption and distribution of a toxic agent to a site distant from its entry point at which point effects are produced. Systemic effects are the obverse of local effects.

Teratogenic -- Causing structural defects that affect the development of an organism; causing birth defects.

Teratology -- The study of malformations induced during development from conception to birth.

Terrestrial – Anything that lives on land as opposed to living in an aquatic environment.

Threshold -- The maximum dose or concentration level of a chemical or biological agent that will not cause an effect in the organism.

Thymus – A small gland that is the site of T-cell production. The gland is composed largely of lymphatic tissue and is situated behind the breastbone. The gland plays an important role in the human immune system.

Toxicity -- The inherent ability of an agent to affect living organisms adversely.

Uncertainty factor (UF) -- A factor used in operationally deriving the RfD and similar values from experimental data. UFs are intended to account or (1) the variation in sensitivity among members of the human population; (2) the uncertainty in extrapolating animal data to the case of humans; (3) the uncertainty in extrapolating from data obtained in a study that is less than lifetime exposure; and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

Vehicle -- A substance (usually a liquid) used as a medium for suspending or dissolving the active ingredient. Commonly used vehicles include water, acetone, and corn oil.

Vertebrate -- An animal that has a spinal column (backbone).

Volatile -- Referring to compounds or substances that have a tendency to vaporize. A material that evaporates quickly.

Xenobiotic – A substance not naturally produced within an organism; substances foreign to an organism.

Xenoestrogen – An estrogen not naturally produced within an organism.

Appendices

Appendix 1 - Worksheet 1 – Analysis for overspray of water with no foliar interception

Appendix 2 – Risks of Ethylene Oxide in NPE Surfactants

Appendix 3 – Toxicity Tables

Table 1 – Toxicity of NP, NPE, NPEC to mammals

Table 2 - Toxicity of NP, NPE, NPEC to fish

Table 3- Toxicity of NP, NPE, NPEC to invertebrates, amphibians, algae

Appendix 4 - WordPerfect Worksheets

Appendix 1 - Worksheet 1
Analysis for Overspray of Herbicides With No Foliar Interception, Using an NPE-based Surfactant Onto Still Water

Assumptions:

- Surfactant is 1% of herbicide mix (typical concentration) (range 0.25% to 2.5%)
- Herbicide mix is typically applied at 25 gallons per acre (gpa) (range 10 to 40 gpa)
- Surfactant is 80% NP9E
- Still water is 3 feet wide, 10 feet long, and 6 inches deep (simulating a pool in a creekbed)

Analysis:

25 gpa x .01 x .80 = 0.20 gallons of NP9E applied per acre (range 0.02 to 0.8 gpa)
3 ft. x 10 ft. / 43,560 square feet/acre = 0.00069 acres in the reach
0.20 gpa x 0.00069 acres = 0.00014 gallons NP9E applied to reach (range 0.000014 to 0.00055 gpa)
3 ft. x 10 ft. x 0.5 ft. = 15 cubic feet of water in reach at any instant
15 cubic feet x 7.48 gallons/ft^3 = 112.2 gallons of water in the pool
0.00014 gal of NP9E / 112.2 gallons water = 1.5 ppm (range 0.15 ppm to 4.9 ppm)

Results:

Instantaneous highest concentration is 1.5 ppm NP9E (range 0.15 to 4.9 ppm)

This result exceeds the protective level for NP9E of 1000 ppb, which would indicate a risk to some aquatic species if the amount of NP9E did not lower through dispersion, sorption, or degradation over time. If we assume that there could be up to 5% of NP and the short-chain ethoxylates (NP1E, NP2E) in the mixture to begin with (refer to section 2.2.1), then the amount of these would be 75 ppb (range of 7.5 to 245 ppb). These values also exceed the chronic standard of protection of 1 ppb for NP and the short-chain ethoxylates.

Drinking Water Exposure to a Child

Assuming a range of drinking water consumption of 0.61 to 1.5 liters per day and a weight of 13.3 kg for a child:

1.5 mg/L x 1 L/day / 13.3 kg = 0.11 mg/kg/day (central)
0.15 mg/L x 0.61 L/day / 13.3 kg = 0.0069 mg/kg/day (lower)
4.9 mg/L x 1.5 L/day / 13.3 kg = 0.55 mg/kg/day (upper)

Appendix 2 – Risks of Ethylene Oxide in NPE Surfactants

Prepared by Patrick Durkin, SERA Inc.

Executive Summary

Surfactants that contain NPE may also contain ethylene oxide. Ethylene oxide is a reasonable concern in the risk assessment of NPE because ethylene oxide is a known carcinogen and the carcinogenic potency of ethylene oxide can be estimated. Based on the high volatility of ethylene oxide, inhalation is primary exposure route of concern. The amount of ethylene oxide released in Forest Service applications involving NPE surfactants is insubstantial relative to other sources of ethylene oxide – i.e., about 1.5 parts in one million. In assessing the risks in localized areas where NPE surfactants are applied, the major uncertainty in the risk assessment of ethylene oxide involves the exposure assessment. No monitoring data are available on concentrations of ethylene oxide in outdoor air associated with the application of NPE surfactants and no exposure models are available that are designed for the exposure assessment of highly volatile chemicals applied in a liquid solution as is the case with ethylene oxide in NPE. Crude approximations of exposure have been made using two very different occupational exposure models: PHED and AGDRIFT. Both of these models yield estimates of exposure indicating that ethylene oxide in NPE does not present a substantial risk to workers, the group of individuals with the greatest potential exposure. At the upper limits of exposure, risks are below the Forest Service trigger level of 1 in one-million. The central estimates of risk, which are more likely to reflect actual risks that workers might be subject to, are in the range of 1 to 8 in 100 million. While confidence in this assessment of risk would be substantially enhanced if direct monitoring data were available, the practical need for such monitoring data (relative to other issues associated with the risk assessment of NPE) does not appear to be compelling.

Assessment

NPE is a complex mixture of nonylphenol ethoxylates that has, as a majority, 8-10 ethoxylate groups attached, along with other longer and shorter-chain NPEs. The available toxicity studies used to support the risk assessment on NPE surfactants involve the technical grade mixtures of the various NPE's rather than pure specific compounds (Section 2.2.1).

Following the general approach recommended by U.S. EPA/ORD (2000), the available data on the toxicity of NPE surfactant mixtures is used directly as the "mixture of concern" and the toxicity data on the technical grade mixture is assumed to encompass the toxicity of impurities in the mixture. While this approach is appropriate for general toxic effects that are considered to have population thresholds, it may not be sufficiently conservative for impurities that may cause cancer, an endpoint that is typically treated as a non-threshold effect.

As noted in Section 2.2.1, NPE is synthesized through a reaction of NP with ethylene oxide and ethylene oxide has been found in NP9E at low levels – i.e., <3.6 ppm to 12.2 ppm – as a residual from the manufacturing process (Johnson 1999). This impurity is a concern to this risk assessment of NPE because ethylene oxide is a carcinogen. As reviewed by ATSDR (1990), various organizations such as the U.S. EPA and the World Health organization have classified the available data on ethylene oxide as "sufficient" in animals and "limited" in humans to assert that ethylene oxide is a carcinogen. More recently, the National Toxicology Program (NTP 2002) has reevaluated the epidemiology data on ethylene oxide and concluded that this compound should be classified as a "known human carcinogen".

Ethylene oxide is a direct-acting alkylating agent. It is this property that is central both to the utility of ethylene oxide in chemical synthesis and to the carcinogenic activity of ethylene oxide. Because it is a direct-acting alkylating agent, ethylene oxide reacts chemically with many large molecules including DNA and the reaction with DNA is the mechanistic basis for both the mutagenicity and carcinogenicity of ethylene oxide (NTP 2002).

While direct acting carcinogens such as ethylene oxide are potentially hazardous by any route of exposure, the physical and chemical properties of ethylene oxide indicate that the primary and probably the only significant route of exposure involves inhalation. Ethylene oxide is highly volatile and is a gas at ambient temperature and pressure. Thus, inhalation exposures are likely. Most of the carcinogenicity studies in experimental mammals have involved inhalation exposures (CalEPA 2002; NTP 2002). Based on the inhalation study by Snellings et al. (1984), the U.S. EPA (1985) derived a cancer potency factor of 0.31 $(mg/kg/day)^{-1}$ and a unit risk factor of 0.000088 $(\Phi g/m^3)^{-1}$. The Snellings et al. (1984) study involved a 2-year inhalation exposure of rats and the potency estimate is based on the development of leukemia in surviving female rats. These cancer potency factors have been reviewed and verified by CalEPA (2002).

Oral and dermal toxicity data on ethylene oxide are extremely limited (ATSDR 1990; WHO 1985), reflecting the difficulty in administering ethylene oxide by these exposure routes. Oral toxicity data appear to be limited to a few gavage studies in which the compound (as a solution in a liquid carrier) is inserted directly into the stomach of the test animals using a special syringe. This is virtually the only way of conducting an "oral" study on a highly volatile compound. One dermal toxicity study on ethylene oxide is available in which ethylene oxide was applied to the skin of mice at a 10% solution over a lifetime (Van Duuren et al. 1965). No skin irritation and no carcinogenic responses were noted. This is probably due to the rapid volatilization of ethylene oxide from the skin.

The limitations in the oral and dermal toxicity data on ethylene oxide do not substantially impact the risk assessment because substantial exposures via these routes are not plausible. Dermal exposures are much less likely because ethylene oxide evaporates rapidly from the surface of skin. While the evaporation rate has not been quantified, it is sufficiently rapid that ethylene oxide will produce a cooling effect that is sufficiently severe to leave burns similar to frost bite (Taylor 1977). While ethylene oxide may occur in foods that have been fumigated, ethylene oxide will either rapidly evaporate from food or react with components in food via alkylation so that foods are not likely to be a significant source of exposure (ATSDR 1990). While ethylene oxide is readily soluble in water, it evaporates rapidly with a half-life of about one hour (Conway et al. 1983).

In assessing the potential risks posed by the presence of ethylene oxide in surfactants used in Forest Service programs, two general questions may be posed:

> Do Forest Service programs contribute substantially to the general contamination of the environment with ethylene oxide?
>
> Does the presence of ethylene oxide in NPE surfactants lead to hazardous levels of exposure in or near areas where NPE surfactants are applied?

As detailed in Table A2-1, the release of ethylene oxide as the result of the use of NPE surfactants in Forest Service programs may be estimated at about 2 lbs/year with a plausible range of about 0.03 lb/year to 15 lbs/year. This estimate is based on the typical application volumes (i.e., the volume of a

field mixture applied per acre) used in Forest Service programs, which ranges from 10 to 40 gallons/acre with a central estimate of 25 gallons per acre. These values are multiplied by the proportion of surfactants in the field mixture (1% with a range of 0.25% to 2.5%) and the proportion of NPE in the surfactant (80%). These proportions are used to calculate the application volume of NPE in units of gallons per acre (AVG_{NPE} in Table A2-1) which is in turn used to calculate the application volume of NPE in units of liters per acre (AVL_{NPE} in Table A2-1). The functional "application rate" of ethylene oxide in units of mg/acre is then calculated as the product of AVL_{NPE} and the concentration of ethylene oxide in NPE in units of mg/L. Note that the actual lower limit of the concentration of ethylene oxide in NPE is zero but a lower limit of 1 mg/L is used in Table A2-1. The functional "application rate" of ethylene oxide in units of mg/acre is then converted to units of kg/acre and then lb/acre. The number of acres treated per year is based on the use of all herbicides, algicides, or plant growth regulators in 2001 (USDA/FS 2002) – i.e., 186257 acres. This assumes that NPE surfactants are used in all applications and this is an overestimate since some applications involve the use of other types of surfactants and some applications do not involve the use of any surfactants.

As reviewed by ATSDR (1990), 1.3 to 3 million pounds of ethylene oxide are produced commercially each year and 10 million pounds of ethylene oxide are released each year from the combustion of hydrocarbon fuels and the release of ethylene oxide from commodity-fumigated materials. In addition, ethylene oxide is a naturally occurring compound that is released from soil by the action of several species of fungi, bacteria, and actinomycetes (ATSDR 1990). The magnitude of this release, however, has not been quantified. Taking only the 10 million pound annual release of ethylene oxide estimated by ATSDR (1990), the estimated annual release of 2 lbs/year (0.03 lb/year to 15 lbs/year) associated with Forest Service programs (Table A2-1) is clearly inconsequential – i.e., equal to or less than 1.5 parts in one million of the total annual release from the combustion of hydrocarbon fuels and commodity-fumigation.

The potential risks associated with the presence of ethylene oxide in NPE surfactants during applications of herbicides or other agents in Forest Service programs is much more difficult to estimate. Ideally, it would be desirable to have monitoring data on concentrations of ethylene oxide in the breathing zone of workers during the application of field solutions containing known concentrations of NPE. This information is not available. It is possible to calculate concentrations in air based on application rates of ethylene oxide using the assumption that ethylene oxide is confined to some limited space above the worker – i.e., instantaneous equilibrium. For a highly volatile compound such as ethylene oxide, however, there is no basis for assuming that ethylene oxide will remain in a confined area around the worker during application. To the contrary, it is most likely that ethylene oxide will volatilize rapidly and disperse so that the concentrations of ethylene oxide in the breathing space of workers will approach zero or ambient background concentrations. While various air dispersion models are available from U.S. EPA (e.g., www.epa.gov/scram001/tt22.htm#screen), these models are designed primarily for point source gas emissions and are not readily adaptable to the release of gases for liquid droplets, the process of concern to the assessment of risks from ethylene oxide in NPE surfactants.

In the absence of monitoring data or directly applicable models, some attempt can be made to better characterizing risk based on two models that are commonly used in exposure assessments for pesticide applicators: PHED and AGDRIFT.

PHED (Pesticide Handlers Exposure Database) is routinely used in U.S. EPA worker exposure assessments and provides a summary of measured exposure rates for both dermal and inhalation exposure workers applying different classes of pesticides by various methods (PHED Task Force, 1995).

This is a synthesis of a large number of occupational exposure studies, which follow generally similar protocols involving measures of dermal deposition using skin patches and measures of inhalation exposures using breathing area air collectors. For the current analysis, a PHED subset was created for workers in backpack applications of herbicides. This subset contains a total of 69 observations of dermal deposition on various parts of the body—e.g., head, neck, chest, and extremities – as well as estimates of the amount of pesticide inhaled. For this data set, total daily exposures were estimated at 258 mg per lb applied with a range of 93.4 to 602.2 mg per lb applied. Inhalation accounted for about 0.04-0.2% of the total potential exposure (i.e., the amount deposited on the skin plus the amount inhaled) in backpack workers. As detailed in Table A2-2, these estimates can be used to calculate the inhalation exposure rate (TER × PI in units of mg/lb ai applied as indicated in row 3). This value can be multiplied by the function application rate of ethylene oxide taken from Table A2-1 to yield a total exposure to ethylene oxide in units of mg (TE in row 5 of Table A2-2). This value can be divided by the body weight to yield the estimated daily dose rate (DDR in row 7 of Table A2-2). The daily dose rate is then multiplied by the cancer potency factor of 0.31 $(mg/kg/day)^{-1}$, discussed above, to yield the estimated risk.

Based on the PHED approach detailed in Table A2-2, the cancer risks range from about 3 in 100 billion to 4 in 10 million with a central estimate of 1 in 100 million. The upper range of risk is based on two very conservative assumptions. First, the cancer potency factor is based on exposure over an entire life span – i.e., about 70 years for humans. Workers involved in strenuous activities such as the application of pesticides are not likely to do so for longer than 30 years and will certainly not apply pesticides every day. The second very conservative assumption involves the calculation of the upper range of exposure, which is based on the assumption that every application involves both the highest application of field solutions (40 gallons/acre in Table A2-1) and the highest proportion of ethylene oxide in the surfactant (0.025 in Table A2-1). While this may yield a plausible estimate of the maximum exposure that a worker might experience in a single day, it is extremely unlikely that a single worker would be so exposed over the course of many years.

An alternative assessment of risk based on the AGDRIFT model is summarized in Table A2-3. AGDRIFT is a model developed as a joint effort by the EPA Office of Research and Development and the Spray Drift Task Force, a coalition of pesticide registrants (Teske et al. 2001). For aerial applications, AGDRIFT permits very detailed modeling of drift based on the chemical and physical properties of the applied product, the configuration of the aircraft, as well as wind speed and temperature. In addition, AGDRIFT estimates one-hour average concentrations of the applied chemical in air adjacent to and downwind from the application site.

AGDRIFT is clearly not designed to model concentrations of ethylene oxide that might be in the breathing zone of a worker applying an NPE surfactant in a ground application. Nonetheless, estimates concentrations in air from AGDRIFT based on an aerial application are likely to be conservative and essentially model the "direct spray" of a worker. A major limitation of AGDRIFT in this application is that AGDRIFT is not designed to handle highly volatile compounds and the chemical and physical properties of ethylene oxide are outside of the range of values permitted by AGDRIFT. In order to obtain an at least crude estimate plausible concentration, AGDRIFT was used to model the application of water. To compensate for the much lower volatility of water compared to ethylene oxide, AGDRIFT was run using a very fine droplet distribution, temperature was set to 90EF, relative humidity was set to 10%, and wind speed was set to one mile per hour. All of these factors will tend to yield high vapor/gas concentration in air. There are a large number of other model parameters – i.e., type of aircraft, nozzle

placement etc. – that have a lesser impact on modeled air concentrations.

The results of the AGDRIFT modeling are presented in Table A2-3. Estimated concentrations in air (1 hour averages) range from 25 to 90 $\Phi g/m^3$ per lb/acre. Multiplying these values by the functional application rates for ethylene oxide (Table A2-1), the estimated concentrations in air range from about 3×10^{-6} to 5×10^{-3}, with a central value of about 9×10^{-4} $\Phi g/m^3$. These estimates are then multiplied by the unit cancer risk of 8.8×10^{-5} $(\Phi g/m^3)^{-1}$ to yield estimated cancer risks from about 3 in 10 billion to 5 in 10 million, with a central value of about 8 in 100 million. As with the corresponding risk estimates based on PHED, the risk estimates based on AGDRIFT are conservative in that they are not corrected for less than lifetime exposure and the upper range of risk is particularly conservative because it assumes that the most highly contaminated formulations are used consistently. As noted above, the exposure assessment is also highly conservative because it assumes that the workers are directly adjacent or in very close proximity to an aerial spray. Workers applying herbicides or other agents by backpack or other ground application methods will not be directly sprayed excepting in accidental/extreme circumstances.

As noted above, neither PHED nor AGDRIFT are designed for the assessment of exposures to highly volatile compounds such as ethylene oxide and this reduces confidence in the risk estimates. Nonetheless, it is somewhat reassuring that these two very different and independent approaches lead to similar assessments of risk, particularly at the upper range of risk. Confidence in this assessment of risk would be substantially enhanced if direct monitoring data were available on concentrations of ethylene oxide in ambient air during the application of pesticides with an NPE surfactant. Given that the central estimates of risk are extremely low and these estimates probably best reflect any underlying risk, the practical need for such monitoring data (relative to other issues associated with the risk assessment of NPE) does not appear to be compelling.

REFERENCES FOR APPENDIX 2

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Table A2-1: Estimated Use and Release of Ethylene Oxide (EO) in Forest Service Programs.

	Typical	Low	High	
Application Volume of Field Solutions (AV_{FS})	25	10	40	gal/acre
Proportion of surfactant in applied solution (P_{Surf})	0.01	0.0025	0.025	Unitless
Proportion of NPE in surfactant (P_{NPE})	0.8	0.8	0.8	Unitless
Application volume of NPE in gal/acre (AVG_{NPE})	0.2	0.02	0.8	gal/acre $AV_{FS} \times P_{Surf} \times P_{NPE}$
Application volume of NPE in liters/acre (AVL_{NPE})	0.757	0.0757	3.028	Liters/acre [AVG_{NPE} × 3.785 L/gal]
Conc. of EO in NPE (C_{EO})	6.6	1	12.2	mg/L [actual lower range is 0]
Application rate of EO in mg/acre ($ARMG_{EO}$)	4.9962	0.0757	36.9416	mg/acre $AVL_{NPE} \times C_{EO}$
Application rate of EO in kg/acre ($ARKG_{EO}$)	5.00e-06	7.57e-08	3.69e-05	kg/acre $ARMG_{EO}/(1000 \times 1000)$
Application rate of EO in lb/acre ($ARLB_{EO}$)	0.000011	1.67e-07	0.0000813	lb/acre $ARKG_{EO}$ × 2.2 lbs/kg
Number of acres treated per year (Acres)	186527	186527	186527	USDA/FS (2002)
EO Release, lbs per year	2.05	0.0311	15.2	$ARLB_{EO}$ × Acres

Table A2-2: Estimated Risk to Workers Applying NPE Surfactants Containing Ethylene Oxide (EO) Based on PHED.

	Central	Lower	Upper	
PHED Total Exposure rate (TER)	258	93.4	602.2	mg/lb ai applied
Proportion via Inhalation (PI)	0.001	0.0004	0.002	Unitless
Inhalation exposure rate (IR)	0.258	0.03736	1.2044	mg/lb ai applied (TER × PI)
App. Rate of EO lbs/acre (AP)	1.10e-05	1.67e-07	8.13e-05	Table A2-1
Total Exposure (TE)	2.84e-06	6.22e-09	9.79e-05	mg/day (IR × AP)
Body weight (BW)	70	70	70	kg
Daily dose rate (DDR)	4.05e-08	8.89e-11	1.40e-06	mg/kg BW/day (TE/BW)
Cancer potency (CP)	0.31	0.31	0.31	(mg/kg/day) -1
Cancer risk (Risk)	1.26e-08	2.76e-11	4.33e-07	Unitless (DDR × CP)
Approximate risk	1 in 100 million	3 in 100 billion	4 in 10 million	

Table A2-3: Estimated Risk to Workers Applying NPE Surfactants Containing Ethylene Oxide (EO) Based on AGDRIFT.

	Central (5 ft)	Lower (10 ft)	Upper (1 ft)	
1 Hour Average concentration rate at 1 to 10 feet	80	40	150	ng/L per lb ai applied,
App. Rate of EO lbs/acre	1.10e-05	7.57e-08	3.69e-05	lbs/acre (kg*2.2)
Air conc. at application rate	8.79e-04	3.03e-06	5.54e-03	ng/L == ug/m3 1,000 L = m3 1,000 ng = ug
Unit risk for cancer	8.80e-05	8.80e-05	8.80e-05	$(ug/m^3)^{-1}$
Cancer risk	7.74e-08	2.66e-10	4.88e-07	Unitless
	8 in 100 million	3 in 10 billion	5 in 10 million	

Appendix 3 - Table 1 - Toxicity of NP, NPE, NPEC to Mammals

Organism	Exposure	Response	Reference
Rats, Rabbits, Mice	Acute exposure to NP9E, no range or timing specified	Oral LD50 (rat) = 1,410 to 5,600 mg/kg Oral LD50 (rabbits, mice) = 620 to 4,400 mg/kg Dermal LD50 (rabbit) = >2,830 mg/kg Minimally to severely irritating to rabbit skin and moderately irritating to rabbit eyes.	WHO, 1998 as cited in Environment Canada, 2001; Smyth and Calandra, 1969
Rats	Acute exposure to NP, no range or timing specified.	Oral LD50 = 580 – 1,620 mg/kg	WHO, 1998, U.S. EPA, 1992a, b, c, as cited in Environment Canada 2001a
Rats, Charles River	Subchronic 90-day exposure to NP9E (tergitol, TP-9) at 0, 10, 50, 250, and 1,250 mg/kg/day; 90-day exposures to NP9E (Igepal CO-630) at 0, 0.01, 0.04, 0.16, 0.64, 2.5, and 5.0% in feed., 90-day exposure to NP9E (Dowfax 9N9) at 0, 0.1, 0.3, and 1.0% in feed.	(Tergitol) 90 day LOEL = 50 mg/kg/day, based on slight reduction of polysaccharides in the liver, NOEL = 10 mg/kg/day (Igepal) 90-day LOEL = 0.64% (~65-71 mg/kg/day); NOEL = 0.16% (~17-20 mg/kg/day) based on weight loss. (Dowfax) 90-day LOEL = 0.3% (~66-75 mg/kg/day), NOEL = 0.1% (~20 mg/kg/day) based on liver, kidney, spleen weights and growth loss.	Three unpublished studies (Shelanski 1960; Mellon Inst. 1959; Dow Chemical Co. 1960) cited in Smyth and Calandra, 1969
Rats, Sprague-Dawley	Subchronic exposure (90 days) to nonoxynol 6 (NP6E) at 0, 40, 200, and 1,000 mg/kg/day	Decrease in weight gain seen at 1,000 mg/kg/day. No effects to hematology or urine chemistry. Increased liver to body weight ratios in males and females at 1,000 and 200 mg/kg and increased kidney to body weight ratio in males at 1,000 and 200 mg/kg (dose related). NOAEL of 40 mg/kg/day. No gross pathological changes; no histopathological changes in any organ examined. No effects to blood chemistry or urine chemistry at any dose. No abnormal behavioral changes in any dose group.	Industrial Bio-Test Labs, Inc. 1963a,. Unpublished studies
Rats, Sprague-Dawley	Subchronic 90 day exposure to NP at levels of 0, 200, 650, 2000 ppm in feed (0, 15, 50, 150 mg/kg). Both males and females. 4 week post-exposure period to see if reversal of any effects occurs. Estrous cyclicity, sperm count, motility, and morphology were examined.	No treatment-related effects to weight gain except in highest dose in both males and females. No mortality or moribundity occurred. No histopathological changes in male or female reproductive organs. No changes to estrous cycle patterns. No changes to weight of reproductive organs. No treatment-related affects to sperm. A significant increase in kidney weight was observed in males at highest dose. NOAEL of 650 ppm (50 mg/kg/day)	Cunny et al, 1997
Rats	Subchronic oral exposure to NP, no range or timing specified.	28 day LOEL (male) = 25 mg/kg; increased liver weights 28 day NOEL (females) = 400 mg/kg (highest dose tested)	Richards 1989, unpublished report as cited in Environment Canada 2001a
Rats, SD	2-year chronic feeding study of NP4E at 0, 40, 200, and 1,000 mg/kg/day in feed.	No mortality. Weight loss at 1,000 mg/kg in both sexes and at 200 mg/kg in females after 12 months but no difference at 24 months.(assumed to be an effect of palatability of food); slight elevation of of liver weight in both sexes at 1,000 mg/kg/day.	Unpublished study cited in Smyth and Calandra, 1969
Dogs, beagles	2 year chronic exposure to NP9E at 0, 8.5, 28 and 88 mg/kg/day; 6-12 month old beagles	No evidence of carcinogenicity. Increase in relative liver weight seen at 88 mg/kg/day while microscopically the livers were normal.	Unpublished study cited in Smyth and Calandra, 1969; Environment Canada 2001a
Dogs, beagles	90-day subchronic exposure to NP9E at 0, 0.04%, 0.64%, and 5.0% in feed (approximately 12, 170, and 1,200 mg/kg/day)	NOEL of 12 mg/kg/day based on decreases in weight gain seen at higher doses. Although it appears that increases in liver weight occur, the only statistically significant effect was the decrease in weight gain.	Shelanski, 1960, cited in Smyth and Calandra, 1969

Dogs, beagles	90-day subchronic exposure to NP4E and NP6E at 0, 40, 200, and 1,000 mg/kg/day	NP4E NOEL = 40 mg/kg/day, based on relative liver weights and emesis during first 3 weeks. NP6E NOEL = 40 mg/kg/day, based on daily emesis noted in first two weeks in dogs receiving 200 and 1,000 mg/kg/day. After first two weeks, only occasional emesis in highest dose group. Slight increase in liver to body weight ratio in females exposed to 1,000 mg/kg/day, but not statistically significant. Gross and histopathologic exams didn't indicate any changes. No abnormalities in hematological studies, blood chemistry, urinalyses, liver function.	Industrial Bio_test Labs, Inc. 1963b and 1963c, Unpublished studies cited in Johnson 1999 and Smyth and Calandra, 1969
Rats, Mol:WIST	Gavage exposure of pregnant rats to NP9E at doses of 0, 50, 250, 500 mg/kg/day or NP30E at 0, 50, 250, 1,000 mg/kg/day on days 6-15 of gestation. Gavage exposure of 500 mg/kg/day NP9E or 1,000 mg/kg/day of NP30E during gestation days 1-20. Dermal exposure to 0, 50 or 500 mg/kg/day NP9E for gestational days 6-15. Also conducted Ames salmonella assay with and without rat liver S9 mix.	NP9E resulted in maternal weight loss at oral 250 and 500 mg/kg/day and decreases in litter size, increase in postimplantation losses. Dose related increases in extra ribs and dilated pelvic cavities in fetuses. Topical NP9E dose of 500 mg/kg/day produced larger litters and lessened postimplantation losses. NP9E not a mutagen based on the Ames assay. NP9E teratogenic and maternal NOAEL is 50 mg/kg/day. No reproductive or teratogenic effects seen with NP30E as well as no maternal weight effects.	Meyer et al 1988
Rabbits	Dermal exposure to NP, no range or timing specified	Dermal LD50 = >2,000 mg/kg Moderately to severely irritating to rabbit skin and eyes	WHO, 1998, U.S. EPA, 1992a, b, c, as cited in Environment Canada 2001a
Mice	NP9E (nonoxynol 9) injected intraperitoneally into 9-10 week old male mice at doses of 20, 40, 50, or 60 mg/kg/day for 5 days. Both positive and negative controls used. 35 days after injections, sperm were analyzed for abnormalities.	The percentage of abnormal sperm was significantly higher in the positive control as compared to negative control and treatment groups. Conclusion was that NP9E did not increase the frequency of morphologically abnormal sperm over that observed in control group.	Buttar et al, 1986
Mice, CD-1	6-week old female mice were given 600 mg/kg/day (gavage) of NP10E (nonoxynol 10) on days 6-13 of gestation.	No mortality in dams. No significant differences from control in number of viable litters, liveborn per litter, % survival, birth weight, and weight gain. NP10E did not induce developmental toxicity in mice.	Hardin et al. 1987
Rats, Sprague Dawley	Multigeneration reproduction study of exposure to NP in diet. Concentrations of 0, 200 ppm (9-35 mg/kg/day), 650 ppm (30-100 mg/kg/day), and 2000 ppm (100-350 mg/kg/day) over three generations.	LOAEL = 200 ppm based on consistent kidney effects in adults (increased weight, renal tubular pathology). NP is a male and female reproductive toxicant at concentrations => 650 ppm. NOAEL for reproductive toxicity set at 200 ppm. Authors conclude that low doses (~12-40 mg/kg/day) appear to pose a greater hazard to the kidneys then to the reproductive system of male or female rats.	Chapin et al, 1999 (also cited as NTP 1997 in Environment Canada 2001a
Rats	Oral (gavage) doses of 2, 10, 50 mg/kg/day of NP. Continuous daily exposure from six-week old males and thirteen-week old females in F0 generation through F1 and F2 generations (after weaning).	No effects to estrous cyclicity or fertility in F0 females. Histopathologic alteration in liver and kidneys of F0 males in the 50 mg/kg dose. FSH increased in F1 at 50 mg/kg dose. NP did not affect AGD or preputial separation in males, while vaginal opening was accelerated in 50 mg/kg dose. No adverse effects to fertility or histopathology of reproductive organs in any offspring. Reproductive NOEL = 50 mg/kg in F0 and 10 mg/kg in F1.	Nagao et al 2001
Rats, Sprague Dawley	NP administered to pregnant F(0) dams at doses of 0, 5, 25, 200, 500, 1000, 2000 ppm in soy and alfalfa-free feed beginning on gestation day 7. F(1) pups maintained on same diet after weaning at PND 21. F(1) pups sacrificed at PND 50.	Severe polycystic kidney disease (PKD) in 100% of male and females at 2000 ppm. At 1000 ppm, 67% of males and 53% of females had mild to moderate PKD. No PKD evident at lower doses in either sex. NOAEL for PKD is 500 ppm (approx. 35 mg/kg/day for dams during pregnancy; 55-60 mg/kg/day for lactating dams and weaning	Latendresse et al 2001

		offspring).	
Rats, Sprague-Dawley	Maternal daily exposures to NP commenced on day 7 of gestation and continued until weaning (3 weeks after birth), oral (100, 250, 400 mg/kg). Males from the F1 generation were orally dosed daily after weaning till mating and sampling at 10 weeks of age.	No offspring from maternal exposure at 400 mg/kg. No mortality effects at any level. Body mass in males reduced in 250 mg/kg dose level. Mean testicular mass was also significantly different between dose levels. Total sperm count was significantly reduced at 250 mg/kg dose. Other measures showed effects to histology of male reproductive system. Sperm count was not significantly affected at 100 mg/kg.	De Jager, Bornman, Oosthuizen, 1999
Rats, Sprague-Dawley	Maternal daily exposure to NP commenced on day 7 of gestation and continued until weaning (3 weeks after birth), in food at 0, 25, 500, and 2,000 ppm. Offspring fed same diet for 55 days. Study intended to look at behavioral effects of NP exposure.	Decrease in maternal food consumption at all NP doses. No effects on sex ratio or number of dead pups per litter. Effects to offspring body weight in both sexes at 2,000 ppm, but only in males at lower doses. There were no behavioral differences noted (open-field activity, play behavior, running wheel, intake of flavored solutions) except in the 2,000 ppm dosed animals, which showed an increase in salt solution intake. Authors suggest that this effect may be related to estrogenic effects.	Ferguson et al, 2000
Rats, Sprague-Dawley	Daily exposures to NP commenced on post natal day 42 in F0 generation; F1 and F2 generations exposed to same diet after weaning. Exposure levels of 0, 25, 200, and 750 ppm in feed. Study intended to look at behavioral effects to females after NP exposure.	Behavioral effects measured using the Morris water maze. There were no significant treatment-related effects on completion of the water maze test in any generation.	Flynn et al, 2002
Rats, Alderley Park (Alpk), Sprague Dawley	Uterotrophic assay in immature Alpk rats, with rats dosed orally with NP for 3 days at levels of 37.5, 75, 150, or 225 mg/kg. Second uterotrophic assay dosed immature Alpk and SD rats orally for 3 days at 250 mg/kg. A third assay dosed ovariectomized Alpk rats orally for 11 days at 100 mg/kg. .	NP administered orally for 3 days showed a significant dose-related increase in uterine weights, with a NOAEL of 37.5 mg/kg/day. In second assay both strains of rats reacted similarly. In the third assay, the dose of 100 mg/kg/day caused a significant increase in uterine weight.	Odum et al, 1999b
Rats, Noble	Uterotrophic assay in ovariectomized rats, with rats dosed orally with NP for 3 days at levels of 45, 75, 150, or 225 mg/kg. Second uterotrophic assay dosed similar rats orally for 3 or 11 days at 150 mg/kg or 11 days at 53 mg/kg. .	NP administered orally for 3 days showed a significant dose-related increase in uterine weights. In second test, dose at 53 mg/kg for 11 days was not uterotrophic (no statistically significant increase in uterine weight over controls). Doses at 150 mg/kg yielded similar results between two tests.	Odum et al, 1999a
Rats, Alderley Park (Alpk),	Mammary gland study – female rats dosed at 0.052 and 37.4 mg/kg/day NP by continuous infusion via a subcutaneous pump for 11 days, or via oral exposure at 100 mg/kg/day for 11 days.	No clinical effects at these doses; no effects on uterus weight or vaginal cytology. No differences in mammary glands between control and treated; no effect on mammary gland cell proliferation (which contradicts earlier study by Colerangle and Roy, 1996). Since they couldn't replicate earlier study showing effects at 0.073 mg/kg/day, they recommend using as the NOEL for estrogenic effects, the results from multigenerational reproductive tests (approx 40 mg/kg/day).	Odum et al, 1999b
Rats, Noble	Mammary gland study – female rats dosed at 0.073 and 53.2 mg/kg/day NP by continuous infusion via a subcutaneous pump.	No clinical effects at these doses; no effects on uterus weight or vaginal cytology. No differences in mammary glands between control and treated; no effect on mammary gland cell proliferation (which contradicts earlier study by Colerangle and Roy, 1996). Since they couldn't replicate earlier study showing effects at 0.073 mg/kg/day, they recommend using as the NOEL for estrogenic effects, the results from multigenerational reproductive tests (approx 40 mg/kg/day).	Odum et al, 1999a
Rats, SD	Several experiments: first, interperitoneal exposure to NP of neonatal male rat pups at 0, 0.08, 0.8, and 8 mg/kg/day from days 1 to 15 after birth. Animals were sacrificed at	In first experiment, NOAEL for male reproductive effects (testis, epididymis, seminal vesicle, and prostate gland weights) was 0.08 mg/kg/day. Liver weights were not affected at any dose, body weight	Lee, 1998

	age 31 days. Second, male pups were given interperitoneal exposure to 8 mg/kg/day from either days 1-18, 6-24, or 13-30. Third experiment involved interperitoneal exposure during days 1-15 at 8 mg/kg/day to male pups, who were then allowed to mature (8-10 weeks) and were subsequently bred to determine effects on reproductive success.	was only affected at highest dose. Effects on testicular development; NP effects are dose dependent with effects seen at 8 mg/kg/day for 15 days beginning at or before 13 days of age, but not if dosed after 13 days of age. In third experiment, reproductive success as an adult was affected. Note that Odum and Ashby 2000 contradicted this study.	
Rats, Alpk (Wistar derived)	Interperitoneal exposure to NP at 8 mg/kg/day during post natal days 1-10	No effects to pup survival; no effects to male reproductive parameters (testis descent, organ weights). No effects to kidney or liver weights.	Odum and Ashby 2000
Rats	Subcutaneous injection of 500 mg/kg/day NP from postnatal days 1 to 5. Effects were evaluated after puberty.	Dysfunction of postpubertal reproductive function in female rats, as well as disrupted development of gonads in both males and females. Male reproductive function was normal. Body weight was affected, although timing of developmental stages was not affected. Non-reproductive behavior was not affected (open field activity and wheel cage activity).	Nagao et al 2000
Rats, Wistar	Oral exposure to NP1EC via drinking water at one dose level (100 µg/L). Female rats were exposed while weaning male rats, from birth through weaning (day 22).	No effects on body weight, testis weight or testis/kidney weight ratio, or testis/body weight ratio.	Sharpe et al, 1995
Rats, Rabbits	Exell surfactant (64% tallow amine, 14% NPEO, 22% EGBE) administered (gavage) to pregnant rats (0, 3, 8, 20, 50 mg/kg/day) on days 7-16 of gestation and rabbits (0, 12, 35, 100 mg/kg/day) for 12 days.	Rat maternal NOEL = 8 mg/kg/day Rabbit maternal NOEL = 12 mg/kg/day No developmental effects seen in rats at any dose (Developmental NOEL > 50 mg/kg/day). Developmental NOEL for rabbits = 12 mg/kg/day (reduced fetal weight gain). No effects on fetal morphology (teratogenic effects) seen in either species.	Munley et al 1996
Rats, Wistar	NP10E administered subcutaneously to 7-week old females at dose levels of 2 and 20 mg/kg/day for 15 weeks. Followed the F1 and F2 generations after this maternal exposure.	Maternal body weight and food consumption increased at 20 mg/kg. No effects to reproductive ability. No effects to fetuses (external, visceral, or skeletal). No effects to physical development or reflexes in F1 generation. No effects seen in the F2 generation.	Aso et al 1999a
Rats, Wistar	NP10E administered subcutaneously to females at dose levels of 5, 20, and 80 mg/kg/day from offspring date of birth to day 21 after birth.	Exposure of offspring via lactation caused no behavioral effects (reflex test, open-field test, water maze test); no effects to physical development or reproductive ability. Effects to growth at highest dose. NOEL of 20 mg/kg/day for growth effects to both dams and offspring.	Aso et al 1999b
Human MCF-7 Cells	*In vitro* exposure of human MCF-7 breast cancer cells to NP at 0.01, 0.1, 1.0, and 10 µM.	Significant increase in growth of MCF-7 cells seen at 1.0 and 10 µM NP (approx. 220 and 2,200 ppm), indicating the estrogenicity of NP.	Blom et al 1998
Human MCF-7 Cells	*In vitro* exposure of human MCF-7 breast cancer cells to OP, NP, NP2EO, NP1EC at concentrations of 0.0001, 0.001, 0.01, 0.1, 1, 10 µM	Significant increase in growth of MCF-7 cells seen with OP at >=0.1 µM, NP at 10 µM, NP2EO at 10 µM, and NP1EC at >=1 µM, indicating the relative estrogenicity of these compounds.	White et al 1994
Humans	Intravaginal application of NP9E for 14 days at 125 mg/day (approximately 2 mg/kg/day)	No effects on blood chemistries, nor on hepatic function.	Malyk, 1981, 1984

Appendix 3 Table 2 -Toxicity of NP, NPE, NPEC to fish

Organism	Exposure	Response	Reference
Flounder, *Pleuronectes americanus*	7 day static test (no renewal) exposures to NP to early life stages, at exposures from 3.7 to 163 µg/L	96 hour LC50 = 17 µg/L (ppb)	Lussier et al, 2000
Silverside, *Menidia beryllina*	7 day flow through test exposures to NP to juvenile fish, at exposures from 20.2 to 195 µg/L	96 hour LC50 = 70 µg/L (ppb)	Lussier et al, 2000
Sheepshead minnow, *Cyprinodon variegatus*	7 day flow through test exposures to NP to juvenile fish, at exposures from 20.2 to 195 µg/L	96 hour LC50 = 142 µg/L (ppb)	Lussier et al, 2000
Sheepshead minnow, *Cyprinodon variegatus*	Exposure to NP. Exposure ranges, lifestage not specified	96 hour LC50 = 310 µg/L (ppb)	CMA Study 8972, 1990 as referenced in US EPA 1996; Naylor 1995
Zebrafish	Exposure to NP. Approximately 110 µg/L (500 nM) for 3 weeks (3-6 weeks of age) during the period of gonad differentiation. Static test.	Vitellogenin induction, although no effect on male/female sex ratios. Males exposed to NP showed reduced reproductive fitness.	Legler et al. 2001, abstract
Zebrafish	Exposure to 10, 30, 100 µg/L NP from hatch until 60 days post-hatch, renewed every 48 hours.	Sex ratios skewed to females at highest dose. Effects to male and female gametogenisis. Effects to hepatocytes but no effects to kidneys (no dose noted).	Weber et al 2001, abstract
Fathead minnow, *Pimephales promelas*	7 day exposure to levels of NP from 0.05 to 3.4 µg/L, during 42 day test of effects to fecundity. No mortality seen at the highest exposure.	NOEC>3.4 µg/L (ppb)	Giesy et al, 2000
Fathead minnow, *Pimephales promelas*	Exposure to R-11 surfactant (NP9E) to establish LC50 value. Exposure range not specified.	96 hour LC50 = 4.0 mg/L (ppm)	Trumbo, 1999
Fathead minnow, *Pimephales promelas*	Exposure to NP. Exposure ranges, lifestage not specified.	96 hour LC50 = 128 – 320 µg/L (ppb)	Various studies, as reported in Servos, 1999, Table 1, rated as a quality 1 or 2 study by the author
Fathead minnow, *Pimephales promelas*	Exposure to NP9E. Exposure ranges, lifestage not specified.	96 hour LC50 = 4,600 µg/L (ppb) 7 day NOEC (survival) = 1,800 µg/L (ppb) 7 day LOEC (survival) = 2,000 µg/L (ppb) 7 day NOEC (growth) = 1,000 µg/L (ppb) 7 day LOEC (growth) = 2,000 µg/L (ppb)	Various studies, as reported in Servos, 1999, Table 1, rated as a quality 1 or 2 study by the author. Dorn et al 1993.
Fathead minnow, *Pimephales promelas*	Flow-through exposure to NP9E, NP1EC.	NP9E 96-hour LC50 = 6,600 µg/L (ppb) NP1EC 96-hour LC50 = 2,000 µg/L (ppb)	Williams et al, 1996, as reported in Staples et al 1998
Fathead minnow, *Pimephales promelas*	Exposure to NP1EC. Exposure ranges, duration, lifestage not specified.	NOEC = 1,000 µg/L	Williams, 1997, as cited in Environment Canada 2001.
Japanese medaka, *Oryzias latipes*	Exposure to NP8.4E, NP, NP1EC and NP2EC. Exposure ranges, lifestages not specified.	48 hour LC50 NP8.4E = 11,600 µg/L 48 hour LC50 NP8.9E = 11,200-14,000 µg/L 48 hour LC50 NP = 1,400 µg/L 48 hour LC50 NP1EC = 9,600 µg/L 48 hour LC50 NP2EC = 8,900 µg/L	Yoshimura, 1986 (Table 6)
Japanese medaka,	Exposure of newly hatched embryos to NP at 0, 0.1, 10, 25, 50, 100 µg/L in static-	Vitellogenin increased after 2 weeks at 100 µg/L, and after 5 weeks at 0.1 and 10 µg/L. Chronic	Kashiwada et al 2001

Oryzias latipes	renewal conditions for 5 weeks. Chronic (230 d.) and two-generation test also.	exposures to 0.1, 1.0 or 25.0 µg/L have little effect on survival. No change in number of eggs, fertilization or hatching ratios at exposures up to 100 µg/L. Abnormalities in second generation seen with exposures at 100 µg/L.	
Japanese medaka, *Oryzias latipes*	Exposure of adult male medaka to NP at rates of 0.03, 0.1 and 0.3 µmol/L (approximately 7, 22, and 66 µg/L). These males were then allowed to mate with unexposed females. Number of eggs spawned and hatching success of eggs was monitored.	Considerable variation in results, so no statistical significance in any results. Authors note a decrease in hatching numbers at the highest exposure, but no differences in other doses. No effect on the number of eggs.	Shioda and Wakabayashi, 2000
Gadus morhua (cod), flounder (*Pleuronectes flesus*)	Adult fish, continuous flow exposures to NP10E. Exposure ranges not specified.	96-hour LC50 (cod) = 2,500 ppb at 15-17°C 96-hour LC50 (cod) = 6,000 ppb at 6-8°C 96-hour LC50 (flounder) = 3,000 ppb at 15-17°C Survival rate and percent normal development for cod eggs and larvae are similar to controls at 200 ppb. In concentrations of <1,000 ppb, cod maintain normal behavior for several months. Fish were more susceptible than bivalves or crustaceans, and within groups, the more active species were more susceptible than sedentary ones.	Swedmark et al 1971, and Swedmark 1976 (as referenced in Lewis 1991)
Rainbow trout (*Salmo gairdneri* Rich.)	12-16 cm long trout exposed to NP8E; flow through test, at concentrations from approximately 4,000 to 10,000 ppb up to 14 days.	24 our LC50 = 5,000 – 6,050 µg/L 96-hour LC50 = 4,120 – 5,350 µg/L 14-day LC50 = 3,970 – 4,540 µg/L	Calamari and Marchetti, 1973
Rainbow trout, *Oncorhynchus mykiss*	Exposure to NP. Exposure ranges, lifestage not specified.	96 hour LC50 = 221 – 270 µg/L (ppb)	Various studies, as reported in Servos, 1999, Table 1, rated as a quality 1 or 2 study by the author
Rainbow trout, *Oncorhynchus mykiss*	Exposure to NP at 10 to 150 ppb for 72 hours, flow through. Males and immature females.	Vitellogenin induction 72-hour EC50 = 14.14 µg/L; induction seen at lowest dose (10 µg/L). 72-hour LC50 = 193.65 µg/L	Lech et al 1996
Rainbow trout, *Oncorhynchus mykiss*	Exposure to NP. Exposure ranges, lifestage not specified	24 hour LC50 = 300 µg/L 96 hour LC50 = 190 µg/L	Dwyer et al 1995, as referenced in US EPA 1996
Rainbow trout, *Oncorhynchus mykiss*	Exposures to NP, NP2E, NP1EC. Female trout exposed to 0, 1, 10, 30, 50 µg/L (ppb) from day of hatch for 22 or 35 days. Monitoring continued for 108 or 466 days.	NP had effects to growth at 30 µg/L over the longer experiment (body weight, length). NP2E had no significant effects at the end of the observed period (466d) although intermediate checks showed decreased weight/length. NP1EC had positive effects to growth at 10 µg/L, but not at 1 or 30 µg/L. No clear pattern of effects was observed. Effects on ovosomatic indexes were either absent or not dose dependent, except for NP at 30 µg/L.	Ashfield, Pottinger, and Sumpter 1998
Bluegill sunfish *Lepomis macrochirus*	Littoral enclosure exposure over 20 days to NP, at mean maximum concentrations of 5, 23, 76, and 243 µg/L. Evaluated over 70 days.	No effects to length or weight at any dose. Using an indirect measure of survival (the effort needed to catch fish), there were no effects seen except at the highest dose. Population captures at the end of the assessment period suggests that survival is affected at 76 and 243 µg/L. Evaluation of activity and behavior also indicate an effect at 243 µg?L. BAF = 87 +-124. NOEC of 76 µg/L; LOEC 243 µg?L.	Liber et al, 1999b
Bluegill sunfish, *Lepomis macrochirus*	Exposure to NP. Exposure ranges, lifestage not specified	96 hour LC50 = 209 µg/L (ppb)	Brooke 1993a, unpublished, as reported in Servos, 1999.
Chinook salmon, *Oncorhynchus tshawytscha)*	Alevins exposure to NP at 100 ng/L, 1, 10 µg/L for 29 days post hatching, evaluated at 103 days post hatching	Examined male and female fish at 103 days post-hatch that were exposed to 10 µg/l NP to evaluate genetic sex vs. gonadal sex. No effects to these fish (genetic sex = gonadal sex). Lower doses not	Afonso et al 2003

		evaluated.	
Atlantic salmon, *Salmo solar*	Exposure to NP. Exposure ranges, lifestage not specified	96 hour LC50 = 130 – 900 µg/L (ppb)	Various studies, as reported in Servos, 1999, Table 1, rated as a quality 1 or 2 study by the author
Atlantic salmon (*Salmo salar*)	Juvenile (approximately 1 year old) salmon exposed to NP (intraperitoneal injection), at 5, 25, 125 mg/kg.	Dose dependent increases in hepatic estrogen receptor (ER) followed by ER-mediated increases in vitellogenin, and zona radiata protein genes.	Yadetie et al 1999
Sheepshead minnow, *Cyprinodon variegatus*	Exposure to NP. Exposure ranges, lifestage not specified	96 hour LC50 = 310 - 460 µg/L (ppb)	Various studies, as reported in Servos, 1999, Table 1, rated as a quality 1 or 2 study by the author
Fathead minnow, *Pimephales promelas*	Exposure to NP. Exposure ranges, lifestage not specified	33 day NOEC (survival) = 23µg/L (ppb) 28 day NOEC (growth) = 23 µg/L (ppb) 28 day NOEC (reproduction) = 7.4 µg/L (ppb)	Ward and Boeri, 1991, as cited in Servos, 1999.
Rainbow trout, *Oncorhynchus mykiss*	Exposure to NP. Exposure ranges, lifestage not specified	90 day NOEC (growth) = 6.0 µg/L (ppb) 90 day LOEC (growth) = 10.3 µg/L (ppb)	Brooke, 1993 as cited in Servos, 1999, Staples et al 1998
Carp, *Cyprinus carpio*	Juvenile flow-through exposure to NP at 1, 5, 10, and 15 µg/L NP over a 70 day period.	NOEC = 1 to 5 µg/L based on changes to blood cells resulting in severe anemia. No changes to liver, kidney or spleen found at any dose.	Schwaiger et al, 2000
Fathead minnows, *Pimephales promelas*	42 day experiment with exposure to various levels of NP (from 0.05 to 3.4 µg/L) occurring on 2nd week, prior to breeding. Egg number, plasma vitellogenin, and plasma estradiol were measured.	Considerable variation in results, no statistically significant results in terms of egg numbers. Results suggest that exposures >0.3-0.4 µg/L may suppress egg production. Similar variability with vitellogenin and estradiol; generally vitellogenin increased in both sexes with NP exposure, but not dose dependent.	Giesy et al, 2000
Fathead minnows, *Pimephales promelas*	42 day exposure of breeding pairs to NP at rates from 0.05 to 3.4 µg/L, and NP9.5E at rates from 0.15 to 5.5 µg/L.	No dose dependent mortality observed. Exposure to NP caused testicular effects, effects to sperm number in males, but not statistically significant. No effects to females. NP9E had no effects on secondary sexual characteristics in either sex at any dose.	Miles-Richardson, et al 1999
Rainbow trout, *Oncorhynchus mykiss*	Flow through exposure to 36.8 µg/L NP, 31.8 µg/L NP1EC, or 38.3 µg/L NP2E for 3 weeks in adult male fish. Second experiment flow through exposure to 0.24, 1.06, 1.5, 5.02, 20.3, 54.3 µg/L NP.	In first experiment, all exposures resulted in increases in vitellogenin and decreases in testicular growth. In second experiment, NOAEL for vitellogenin induction = 5.02 µg/L. No significant effect to gonadosomatic index until 54.3 µg/L.	Jobling et al, 1996
Rainbow trout, *Oncorhynchus mykiss*	*In vitro* exposure of male trout hepatocytes to NP, NP2E, and NP1EC at three rates each to measure vitellogenin production.	With NP, vitellogenin induced at 220 µg/L, not at 22 µg/L. NP2E induced at 308 µg/L, not 30.8 µg/L. NP1EC had slight inducement at 278 µg/L, but at lower level than corresponding amounts of induced vitellogenin of NP at 220 µg/L. Would appear that in this test, NP > NP1EC = NP2E as far as ability to induce vitellogenin.	White, et al, 1994
Rainbow trout, *Oncorhynchus mykiss*	Flow through exposure to NP at levels from 0.25 to about 55 µg/L for 21 days. Female juvenile trout.	Increase in vitellogenin, NOEC = 6.7 µg/L; LOEC = 16 µg/L. Increase in relative liver weights at >= 19 µg/L, consistent with vitellogenin increase. No effects to body weight or relative weight of gonads at any dose.	Thorpe et al 2000
Eelpout, *Zoarces viviparus*	Intraperitoneal injection of NP into sexually mature male eelpout (a teleost fish), twice a week for 25 days (100 µg/g per week). A second experiment included an additional dosing of NP at 10 µg/g per week, along with 0.5 µg/g per week of estradiol. A third experiment involved seawater with 1 mg/L NP for 3 weeks (changed 3 times).	All three experiments resulted in significant increases in vitellogenin. No changes to liver size, but decreases in weight of testes in relation to body size in all doses tested., effects on germ cells.	Christiansen, Korsgaard, Jespersen 1998

Appendix 3 - Table 3 - Toxicity of NP, NPE, NPEC to Invertebrates, amphibians, algae

Organism	Exposure	Response	Reference
Boreal toad, *Bufo boreas*	Exposure of tadpole to NP, exposure range not specified.	96 hour LC50 = 120 µg/L (ppb)	Dwyer et al, 1997, as reported in Servos, 1999.
Frog, *Rana catesbiania*	Exposure of tadpole to NP, exposure range not specified.	10 day (sediment) LC50 = 260 mg/kg	Naylor, 1995
Frog, *Rana catesbiania*	Exposure of tadpole to NP, exposure range not specified, 30 day exposure period with dosed sediment.	30 day LC50 = 260 mg/kg 30 day NOEL = 155 mg/kg	CMA Study 8981, 1992 (Ward and Boeri, 1992), as referenced in US EPA 1996 and Servos 1999.
Frog, *Rana catesbiania*	Exposure of tadpole to NP, exposure range not specified..	30 day (sediment) LC50 = 260 mg/kg 14 day (interstitial water) LC50 = 75 µg/L (ppb) 14 day (closed water) LC50 = 119 µg/L (ppb)	Weeks et al 1996, as cited in Servos, 1999
Frog, *Xenopus laevis*	Exposure of embryos to NP for 14 days (stage 60 to stage 66), evaluating effects on tail resorption.	14 day NOEC = 25 µg/L 14 day LOAEL = 50 µg/L Authors assumed effects seen (reduced tail resorption) were caused by impacts to thyroid	Fort, Stover 1997; Fort, Propst, Stover, 1998
Frog, *Xenopus laevis*	Exposure of tadpoles to NP in water for 12 weeks (refreshed 3X weekly) at rates of 22 µg/L or 2.2 µg/L (10-7 Molar and 10-8 Molar).	Significant increase in percentage of female frogs at the 22 µg/L exposure.	Kloas, Lutz, Einspanier, 1999
Three frog species, *Xenopus laevis, Crinia insignifera, Litoria adelaidensis*	Exposure of embryos to NP8E, 0.5 to 10 mg/L, over at least 96 hours	X. laevis 96 hour LC50 = 3.9 to 5.4 mg/L (ppm) X. laevis 96 hour developmental EC50 = 2.8 to 4.6 mg/L (ppm) X. laevis minimum con'c to inhibit growth (MCIG) = 1.0 mg/L (ppm) L. adelaidensis 140 hour LC50 = 9.2 mg/L L. adelaidensis 140 hour developmental EC50 = 8.8 mg/L (ppm) L. adelaidensis MCIG = 2.0 mg/L C. insignifera 134 hour LC50 = 6.4 mg/L C.insignifera 134 hour developmental EC50 = 4.5 mg/L (ppm) C. insignifera MCIG = 4 mg/L (ppm)	Mann and Bidwell, 2000
Six frog species, *Xenopus laevis, Bufo marinus, Crinia insignifera, Heleioporus eyrei, Limnodynastes dorsalis, Litoria adelaidensis*	Exposure of feeding-stage tadpoles to NP8E, static renewal test, range of rates not specified. Also repeated with B. marinis for 96 hours at high water temperatures and 12 hours at high water temperatures combined with low dissolved oxygen. Intent was to measure narcosis (either mild or full). Exposure lasted 48 hours.	X laevis was most sensitive species, with EC50 0f 1.1-1.2 mg/L for mild narcosis and 2.3 to 2.8 mg/L for full narcosis. Range for other species 2.7 to <10.6 mg/L for mild; 3.5 to 12.1 mg/L for full narcosis. High temperatures resulted slight change in 96-hour EC50 levels, from 2.8 –5.4 mg/L at lower temp to 3.5 to 4.0 mg/L (mild to full narcosis). High temps plus low dissolved oxygen reduced 12 hour EC50 from 3.6 to 4.1 mg/L to 1.8 and 1.8 mg/L (mild to full narcosis). Tadpoles did exhibit some recovery from narcosis over life of tests (non-significant).	Mann, Bidwell 2001
Freshwater clam, *Anodonta cataractae*	Static test with NP over 144 hours	144-hour EC50 = 5,000 µg/L	McLeese et al, 1980
Clam, *Mulinia*	7 day static test (no renewal) exposures to	96 hour LC50 = 37.9 µg/L (ppb)	Lussier et al, 2000

lateralis	NP to embryo life stages, at exposures from 2.4 to 800 µg/L		
Grass shrimp, *Paleomonetes vulgaris*	7 day flow through test exposures to NP to larval shrimp, at exposures from 20.2 to 195 µg/L	96 hour LC50 = 59.4 µg/L (ppb)	Lussier et al, 2000
Mysid	Exposure to NP, exposure range not specified	28 day NOEC (survival) = 6.7 µg/L (ppb) 28 day NOEC (reproduction) = 6.7 µg/L (ppb) 28 day NOEC (length) = 3.9 µg/L (ppb)	Naylor 1995; Staples et al 1998
Mysid	Exposure to NP9E, exposure range not specified	48 hour LC50 = 900 - 2,000 µg/L (ppb)	Hall et al, 1989
Mysid (*Mysidopsis bahia*)	48 hour static renewal exposure to NP9E	48-hour LC50= 1,230 µg/L (ppb)	Patoczka and Pulliam, 1990
Marine copepod *Tisbe battagliai*	Exposure to NP over 53 days at 20, 41, 74, and 301 µg/L.	Exposures at 74 and 301 µg/L resulted in no survival; exposure to 41 µg/L resulted in little survival. There were no significant differences between controls and 20 µg/L. There was a tendency for a reduced intrinsic rate of natural increase in population, but not significant. No effects to sex ratios in offspring.	Bechmann, 1999
Amphipod, *Leptocheirus plumulosos*	7 day flow through test exposures to NP to adult, at exposures from 20.2 to 195 µg/L	96 hour LC50 = 61.6 µg/L (ppb)	Lussier et al, 2000
Amphipod, *Hyalella azteca*	Exposure to NP, exposure range not specified	96 hour LC50 = 20.7 – 170 µg/L (ppb)	Brooke 1993, and England and Bussard, 1994, as reported in Staples et al 1998.
Daphnia magna, Chironomus spp. (midge), *Hyalella azteca, Stagnicola elodes* (pond snail), *Nephelopsis obscura* (leech)	Exposure to X-77 spreader surfactant, both as a tank mix with Rodeo in the field (approx ½%) and in lab.	Daphnia 48 hour LC50 = 2.0 mg/L (1.5-2.7) Hyalella azteca 96 hour LC50 = 5.3 mg/L (4.3-6.7) Chironomus riparius 48 hour LC50 = 10.0 mg/L (8.2-13.1) Nephelopsis obscura 96 hour LC50 = 14.1 mg/L (10.7-19.0) X-77 was about 100 times more toxic than Rodeo. Daphnids were more sensitive then other species to X-77, Rodeo, and Chem-Trol (drift reducer). Mixtures were generally additive in toxicities. Field application of mixture over wetlands did not cause mortality to tested species above controls.	Henry, Higgins, Buhl 1994
Chironomidae, Oligochaeta, Mollusca	Littoral enclosure exposure over 20 days to NP, at mean maximum concentrations of 5, 23, 76, and 243 µg/L.	Population abundance at 5 and 23 µg/L not affected while some differences at 76 µg/L were significantly different than controls, and at 243 µg/L were often significantly different. The NOEC for the most sensitive taxa is a mean maximum concentration of 23 µg/L (an average of 14 µg/L). Adult emergence of Chironomidae was reduced during application and increased after. The effect is likely due to the surfactant effect of NP, reducing surface water tension and preventing emergence.	Schmude et al 1999
32 taxa of zooplankton in Cladocera, Copepoda, and Rotifera	Littoral enclosure exposure over 20 days to NP, at mean maximum concentrations of 5, 23, 76, and 243 µg/L.	Reductions in abundance seen in most sensitive taxa at a mean maximum concentration of 23 µg/L NP (Calanoida, Herpacticoid). Although some taxa were affected at this level, the overall zooplankton community structure was relatively unaffected using the Shannon-Wiener diversity index. The MATC (maximum acceptable toxicant concentration) was estimated at approximately 10	O'Halloran et al 1999

		µg/L.	
Amphipod, *Eohaustorius estuaries*	96-hour static exposures to NP, at 0, 10, 120, 200, 360, and 600 µg/L	96 hour LC50 = 189-299 µg//L (ppb); authors also noted a recovery time for normal reburial in clean sediment/water after exposures above 10 µg/L	Hecht and Boese, 2002
Lobster, *Homarus americanus*	7 day static test (renewed) test exposures to NP to zoea first stage, at exposures from 16 to 330 µg/L	96 hour LC50 = 71 µg/L (ppb)	Lussier et al, 2000
Dyspanopius sayii	7 day flow through test exposures to NP to zoea 4th and 5th stage, at exposures from 20.2 to 195 µg/L	96 hour LC50 = >195 µg/L (ppb)	Lussier et al, 2000
Dragonfly, *Ophiogomphus spp.*	Exposure to NP, exposure range and lifestage not specified	96 hour LC50 = 596 µg/L (ppb)	Brooke 1993, as cited in Servos 1999.
Snail, *Physella virgata*	Exposure to NP, exposure range and lifestage not specified	96 hour LC50 = 774 µg/L (ppb)	Brooke 1993, as cited in Servos 1999.
Snail, *Lymnaea stagnalis*	Exposure to NP at 1, 10, 100 µg/L for 7 weeks and 100 µg/L for 12 weeks; recently matured adults.	7-week exposure resulted in no effects to egg production, hatching rate of eggs; shell height and weight of adults; mortality, or histopathology. Exposure for 12 weeks esulted in a decrease in fecundity as well as histopathological changes to the epithelial tissues of the adults (e.g., lung and foot characterized by extreme inflammatory processes). There were no differences in egg laying rates of the F1 generation.	Czech et al, 2001
Daphnia, *Daphnia spp.*	Exposure to NP, exposure range not specified	48 hour LC50 = 140 – 190 µg/L (ppb) NOEC (reproduction) = 24 – 116 µg/L (ppb)	Various studies, as reported in Servos, 1999, Table 1, rated as a quality 1 or 2 study by the author and as reported in Environment Canada 2001a
Daphnia, *Daphnia magna*	Exposure to NP and NP9E, exposure range not specified.	NP 48 hour LC50 = 190 µg/L NP9E 48 hour LC50 = 14,000 µg/L	Naylor 1995
Daphnia, *Daphnia magna*	Acute toxicity test of NP at 32, 56, 100, 180, 320, and 560 µg/L 21-day chronic exposure to NP, 0, 14, 24, 39, 71, 130, 250 µg/L	24 hour EC50 = 300 µg/L (ppb) 48 hour EC50 = 190 µg/L (ppb) 21 day NOEC (reproduction) = 24 µg/L (ppb) 21 day NOEC (length) = 39 µg/L (ppb)	Comber, et al, 1993
Daphnia galeata	Exposure to NP; acute and a two generation life table test (0, 3, 10, 30, 50, 70, 100 µg/L) with a 45-day duration	48-hour EC50 (immobility) = 60-67 µg/L Chronic 45-day NOAEL = 30 µg/L 45-day EC50 (population intrinsic rate of increase) = 65.2 µg/L	Tanaka and Nakanishi, 2002
Daphnia, *Daphnia magna*	Exposure to NP, exposure range not specified.	35-day chronic exposure to 50 µg/L increased fecundity and neonate deformities, but no change in sex ratios. 3-day exposure at 200 and 800 µg/L caused neonate deformities. Exposure at 1,000 µg/L arrested embryo development.	Zhang and Baer, 2001
Daphnia, *Daphnia magna*	Exposure to NP to embryos at levels of 0.46µM (100 µg/L) and 0.91µM (200 µg/L) for three weeks. To determine LC50, embryos were also exposed to a series of concentrations from 31 µg/L to 163 µg/L. (time element not specified)	NP is embryotoxic, threshold concentration is approx 44 µg/L (7 day?)	LeBlanc, Mu, and Rider 2000
Daphnia, *Daphnia galeata*	Exposure to NP at 10, 50, 100 µg/L for 30 days; earlier experiment exposed Daphnia to 150 µg/L for 48 hours	100% mortality when exposed to 150 µg/L for 48 hours. Number of male offspring not affected at 10, 50 or 100 µg/L, but number of females increased with exposure, although not dose dependent. Deformities seen in offspring when prenatally exposed to nonylphenol; which appears	Shurin and Dodson 1997

		to be dose dependent (11% of offspring at 10 µg/L).	
Daphnia, *Daphnia spp.*	Exposure to NP2E and NP2EC, exposure range not specified	NP2E 48 hour LC50 = 115 - 198 µg/L (ppb) NP2EC 48 hour LC50 = 770 – 1,295 µg/L (ppb)	Maki et al, 1998
Daphnia, *Daphnia spp*	Exposure to NP9E, exposure range not specified	48 hour EC50 = 14,000 µg/L (ppb) 7 day LC50 (mortality) = 10,000 µg/L (ppb) 7 day NOEC (growth) = 10,000 µg/L (ppb)	Dorn et al 1993; Naylor 1995; Staples et al 1998
Midge, *Chironomus tentans*	Life-cycle test with exposure to NP. Problems with design, such that NP dose was not constant over 53 days of test (intent was for 12.5, 25, 50, 100, 200 µg/L), but considered constant for first 18 days.	20 day NOEC = 42 µg/L (ppb) 20 day LOEL = 91 µg/L (ppb) No effects to larval growth, survival past 20 days, emergence success or pattern, sex ratio, fecundity, or egg viability at any level tested.	Kahl et al, 1997
Earthworm, *Apporectodea calignosa*	Exposure to NP in soil, exposure range not specified.	21 day EC10 (reproduction) = 3.4 µg/g in soil	Krogh et al, 1996 as cited in Environment Canada 2001a.
Duckweed, *Lemna minor*	Exposure to NP, exposure range not specified	LOEC = 2,080 µg/L (ppb) NOEC = 901 µg/L (ppb)	Brooke1993, as reported in Staples et al 1998
Green algae, *Selenastrum capricornutum*	Exposure to NP, exposure range not specified	96 hour EC50 = 410 µg/L (ppb) 96 hour NOEC (biomass) = 92 – 694 µg/L (ppb)	Brooke, 1993, as cited in Servos, 1999; Naylor, 1995
Green algae, *Selenastrum capricornutum*	Exposure to NP9E, exposure range not specified	96 hour EC50 (growth) = 12,000 µg/L (ppb) 96 hour NOEC (growth) = 8,000 µg/L (ppb)	Dorn et al, 1993; Naylor 1995.
Marine algae, *Skeletonema costalum*	Exposure to NP, exposure range not specified	96 hour EC 50 (growth) = 27 µg/L (ppb) NOEC = 10 µg/L (ppb)	Naylor 1995

Appendix 4 – Worksheets – Hardcopy only – not electronic